BACTERIA IN A PINE FOREST SOIL.

BIOMASS AND ACTIVITY AS AFFECTED BY ENVIRONMENTAL FACTORS.

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Abstract

The dynamics of soil bacteria in a pine forest soil were investigated in relation to clear-cutting and environmental factors.

A method was developed, using fluorescein diacetate (FDA) as the stain, for counting metabolically-active soil bacteria in the fluorescence microscope. Acridine orange (AO)-staining was used for estimating total counts of soil bacteria. The magnification of the microscope used was shown to affect estimated bacterial counts, and the method used to obtain average cell volumes greatly affected the estimated biovolume. It was thus recommended to carefully standardize the procedures for obtaining comparable results.

Initially, the bacterial biomass increased after clear-cutting compared to a mature pine stand. The biomass then progressively decreased. In plots receiving cutting residues constantly greater biomass was found compared to plots without residues.

The bacterial numbers covaried in the three areas investigated (mature pine stand and two clear-cut treatments) during three years with monthly samplings. However, no seasonal fluctuations repeated every year could be detected. The bacterial numbers seemed to be susceptible to short-term changes in environmental factors. The AO-stained bacterial numbers were correlated to moisture content, while FDA-stained bacterial numbers were correlated to precipitation. This indicate a greater susceptibility of FDA-stained bacteria to rapid changes in the environment. The bacterial numbers were also positively correlated to nematode numbers.

In laboratory experiments the presence of bacterial-feeding nematodes greatly stimulated bacterial activity measured as respiration. However, at great nematode densities the bacterial numbers were depressed.

Key words Soil bacteria, biomass, biovolume, coniferous forest, acridine orange, FDA, magnification, clear-cutting, cutting residues, soil moisture, precipitation, nematodes, Acrobeloides, direct counting, fluorescence microscope.

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PAPERS I - V

This dissertation is based on the following publications, referred to in the text by Roman numerals, and on some unpublished results.

I. LUNDGREN, B. (1981) Fluorescein diacetate as a stain of metabolically active bacteria in soil. Oikos 36, 17-22.

II. LUNDGREN, B. Effects of size classification on microscopically estimated biovolumes of soil bacteria. Submitted to Soil Biol. Biochem.

III. LUNDGREN, B. (1982) Bacteria in a pine forest soil as affected by clear-cutting. Soil Biol.Biochem. 14, 537-542.

IV. LUNDGREN, B. and SÖDERSTRÖM, B. (in press) Bacterial numbers in a pine forest soil in relation to environmental factors. Soil Biol. Biochem.

V. LUNDGREN, B. and SOHLENIUS, B. Impact on soil bacterial numbers and activity by a Cephalobid nematode. Submitted to Microb. Ecol.

INTRODUCTION

In the decomposition of organic matter in soil almost all the carbon passes through the microorganisms (Reichle 1977). In a pine forest with a podzolic soil profile in Central Sweden about 60 % was calculated to pass through the bacterial populations (Persson et al. 1980). However, the bacterial metabolic activity and growth in soil are influenced by several environmental factors.

Bacterial growth takes place in aqueous solution only. These organisms are thus affected by the variation in water content of the soil (Tate, III and Terry 1980, Campbell and Biederbeck 1976). In dry soil there is not only a spatial effect of decreased water content, but also an effect of lowered water potential caused by increased osmotic and matric forces. This in turn lowers the metabolic activity of the bacteria (Wilson and Griffin 1975). The activity is of course also dependent on the soil temperature. An increase in temperature generally results in increased activity. This is reflected in the Q_{10}-values of respiration, which are normally about 3 (Bunell et al. 1977).

The bacterial activities are affected by nutrient availability, too. There are three main nutrient sources, which fluctuate both seasonally and on a short term basis due to climatic variations. These nutrient sources are above ground litter (Flower-Ellis and Persson 1980), root litter (Persson 1980b), and root exudates (Bowen and Theodorou 1973). Other biotic factors are also of

importance in regulating the bacterial populations. Competition between microbial populations probably plays an important role in this regulation (Frederickson and Stephanopoulos 1981). Predation by the bacterial feeding microfauna probably exerts a considerable influence on the bacteria (Alexander 1977). Among these animals, protozoa and nematodes are the most important ones (Anderson et al. 1978).

In a natural undisturbed ecosystem the dependent environmental factors (e.g. microbial and faunal populations) balance each other, and adapted to fluctuations in the independent factors (e.g. moisture content and temperature) normally occurring. If the ecosystem is disturbed by natural causes, e.g. forest fire, or by human activities, e.g. clear-cutting, most of the above mentioned environmental factors will be affected. Nutrient availability may be changed, since there is no longer any litter production and root exudation from the trees. In some cases new vegetation, like grasses, may develop (Ingelög 1974), which creates new qualitatively different nutrient sources for the microorganisms. The microfaunal community may be changed (Huhta 1976), which in turn changes the predation pressure on the microbial populations.

To study the dynamics of the soil bacteria in relation to environmental factors and ecosystem disturbances, there is a need for proper techniques to quantify the bacteria. A great number of methods are available. Different plate counting methods have been developed. Among

others, standard laboratory media, often diluted (Martin 1975, Hattori 1976), or any kind of soil extract media (see Parkinson et al. 1971) have been used as substrates.

A variety of techniques can be designated as direct counting methods. An advantage of most of these methods is that they do not have selective features as plate counting methods (Parkinson et al. 1971). As a consequence, direct counting methods generally result in much higher counts than do the plate counting techniques (Babiuk and Paul 1970, Trolldenier 1973). Transmission electron microscopy has been used (Nikitin 1973, Balkwill et al. 1977) to obtain as high a resolution as possible because of the small sizes of soil bacteria. Today the most widespread techniques for direct counting make use of fluorochrome staining of the microorganisms followed by counting in the fluorescence microscope. With these methods it is possible to distinguish easily between bacteria and soil particles (Babiuk and Paul 1970, Anderson and Slinger 1975).

In this thesis a new method for direct microscopical counting of metabolically active bacteria is described, and some problems in estimating soil bacterial biomasses in the microscope are discussed. In a field investigation of clear-cutting of a pine forest the effect on bacterial populations in a podzolic soil was studied. The fluctuations in bacterial numbers obtained were registered in relation to some environmental factors. The effects of bacterial feeding nematodes were studied in more detail in laboratory experiments.

METHODOLOGICAL STUDIES

Direct microscopical counting of the bacteria was used throughout the work presented in this thesis. To obtain total counts and biomasses in the field investigations, fluorescence microscopy of soil smears was used after staining with acridine orange (AO, Trolldenier 1972, as modified by Clarholm and Rosswall 1980). In the laboratory experiments in papers I and V a membrane filter technique was used for the AO-stained preparations (described in paper I). The AO-stained bacterial counts include both living and dead bacteria.

Obviously, it is often of interest to estimate the biomass of metabolically active organisms. A method was thus developed for this purpose (paper I). The stain used in this method, fluorescein diacetate (FDA), is a fluorogenic substance which does not fluoresce. After enzymatic hydrolysis to fluorescein by e.g. esterases or proteases, light emission will occur after excitation in light of short wavelength. Since fluorescein accumulates in living cells (Rotman and Papermaster 1966) the glowing of bacterial cells under the fluorescence microscope indicates enzymatic activity within the cells. FDA-stained bacterial counts can thus be used for estimation of numbers of metabolically active bacteria (paper I).

Estimation of bacterial biomasses by direct microscopical techniques is usually carried out in three steps. 1) The bacterial numbers, and 2) the average cell volumes

are estimated. These two steps give the total bacterial biovolume. 3) The biovolume is multiplied by a volume--weight conversion factor to obtain the biomass of bacteria, in most cases the dry weight. Several sources of error occur in this process. Some of them were studied in AO-stained preparations.

Firstly, the effect of using different microscopical magnifications in obtaining bacterial numbers, was investigated. Three magnifications were included: 320x, with 40x objective, NA 0.90 and 8x eyepieces; 800x, with 100x oil immersion objective, NA 1.25 and 8x eyepieces; and 1250x with the same objective but 12.5x eyepieces (wide angle). On three occasions five soil samples were taken from the organic soil layer of a podzol (see paper II). Counts were made in each sample with all three magnifications. Highest bacterial counts were obtained by the 800x magnification (3.2×10^{10} g^{-1}dw) followed by 1250x (2.6×10^{10} g^{-1}dw) and 320x (1.3×10^{10} g^{-1}dw). These differences were found to be significant ($p < 0.001$) in a two-factor ANOVA, with magnification and sampling date as the two factors. The low value of the lowest magnification was probably due to insufficient magnification to allow the eye to detect the smallest bacteria. From this point of view the comparably low value of the greatest magnification was unexpected. However, with the equipment used, these fields of view appeared less bright and the resolution appeared worse. Thus, it was difficult to systematically examine each field of view, and some bacterial cells were probably missed in the counts. This

emphasizes the importance of testing the equipment to obtain optimal results. In the following investigations the magnification of 800x was used.

In another study, described in paper II, three techniques to estimate the average cell volume were compared, namely measurements of individual bacterial cells, and two size classification methods. Differences of up to five times in average cell volume obtained, were depending on which method was used. Often size classification is used, since measurement of individual cells is very time-consuming. The results emphasize the importance of selecting proper class limits for the size classification in each investigation (paper II). The results thus obtained should then be compared to measurements of individual cells in a pilot study to allow for adjustments of the class limits.

The last step in biomass estimations is the conversion of volumes to weights. The literature gives different values for the volume-weight conversion factor, and in reality some variation in this factor does occur because of fluctuations in nutrient availability and moisture regimes (van Veen and Paul 1979). But, this variation is probably not as great as the variation in the literature data. A method often used to obtain a conversion factor for fresh weight is density gradient centrifugation in CsCl. The resulting densities range from 1.0 to 1.8 g cm^{-3} (Parkinson et al. 1971). In most cases pure cultures have been used. Faegri et al. (1977) used soil suspensions and obtained a density of 1.3 g cm^{-3} of the bacteria.

They used a dry weight of 20 % of wet weight and thus got a conversion factor of 0.26. Martin and MacDonald (1981) got a wet weight density of 1.081-1.123 g cm^{-3} with density gradient centrifugation in inert Percoll. Unfortunately, they did not estimate the dry weight. Van Veen and Paul (1979) obtained dry weight specific gravities of bacteria and yeasts from 0.38 to 1.4 g cm^{-3} with an average of 0.8 g cm^{-3}. Differences of at least four times in the volume-dry weight conversion factor can thus be found.

Only by using different combinations of methods for average cell volume estimations and volume-weight conversion factors, discrepancies in biomass estimations of up to one order of magnitude can easily be obtained. An example of this is shown by comparing the investigations in paper III and the one by Clarholm and Rosswall (1980). In paper III a dry weight of 3.4×10^{-14} g $bacterium^{-1}$ (conversion factor 0.26) was found in the organic soil layer, while the mean dry weight (calculated from their mean values of bacterial numbers and biomass) in Clarholm and Rosswall (1980) was 2.4×10^{-13} g $bacterium^{-1}$. They used a volume-dry weight conversion factor of 0.20. Assuming the same bacterial numbers the latter investigation would give a biomass 7.1 times greater than the former.

The different examples discussed above of possible errors in obtaining biomass values of soil bacteria from direct microscopy emphasize the difficulties in using the results as absolute values. The great value of these

methods is for comparable purposes, e.g. to follow the dynamics of the bacterial populations with time or to compare data from different soil treatments. Great precision can be attained when using a carefully standardized technique.

FIELD STUDIES

The site was a Scots pine (Pinus sylvestris L.) forest at Ivantjärnsheden in the province of Gästrikland, Central Sweden. This was the main research site of the Swedish Coniferous Forest Project, and detailed descriptions of the site area are given by Axelsson and Bråkenhielm (1980) and Persson (1980a). The soil was an iron podzol on glacifluvial sandy sediment. The soil layers sampled in the investigations were the organic fermentation/humus (A_{01}/A_{02})-layer and two mineral soil layers, the bleached (eluvial, A_2) soil layer and the enrichment (illuvial, B) soil layer.

A rather sparse vegetation was found below the tree layer. The shrub layer was of very small significance. The field layer consisted mainly of heather (Calluna vulgaris L.) and lingonberry (Vaccinium vitis-idaea L.). In the bottom layer were found lichens and mosses like Cladonia spp. and Pleurozium sp., respectively.

Samples were taken in three areas. IhVA was a mature pine stand about 120 years old. This area was used as a reference stand. Ih0 was clear-cut in March-April 1976, and divided into two treatments. In one the cutting

residues were removed, while in the other one double the normal amount was left on the ground. The field layer almost completely disappeared, and detrimental effects on the bottom layer were also found, especially in the treatment without residues. The microclimate in the clear-cut area was changed, which together with the vegetational changes altered the chemical and physical properties of the soil. An additional clear-cut are (IhI) was also investigated. This was clear-cut in 1967. Large areas at this place were bare, completely lacking vegetation. In some patches, growth of pines was established.

The bacterial populations were followed with monthly counts three years at the clear-cut area, Ih0 (see papers III and IV). The samplings started directly after cutting in both treatment areas, with and without slash (cutting residues), respectively. For comparison, samples were also taken at the old clear-cut area (IhI) and the mature pine stand (IhVA). Additional samples were taken in the following two years.

In the organic soil layer (A_{01}/A_{02}) the bacterial biomass increased at Ih0 compared to IhVA during the first two years, after which a decrease was found (see Fig. 2 in paper III). The low values obtained at IhI indicated that this decrease in biomass may continue for several years. Similar results were found in the mineral soil layers (A_2 and B) although they were not as obvious as in the organic soil layer. Approximately the same results were found in FDA- and AO-stained bacterial biomasses. Several explanations for the changes are possible. Mycorr-

hizal fungi may suppress the saprotrophic microorganisms. Because of ceased root growth the mycorrhiza probably disappeared after clear-cutting, which reduced the suppressive effects (Gadgil and Gadgil 1978). This may explain the increase in bacterial biomass during the first year. Since root production and root exudation ceased, due to absence of trees and growth of ground vegetation, this eventually led to decreased nutrient availability. This in turn may have caused the decrease in bacterial biomass.

The higher biomass values in the treatment with slash on the ground compared to the one without were probably not due to addition of nutrients by the slash, but to a higher moisture content of the soil in this treatment.

The monthly fluctuations in FDA- and AO-stained bacterial numbers were similar in the two treatments at Ih0 and IhVA (paper IV). During the three years investigated, no seasonal fluctuations could be detected, however. The fluctuations seemed to be due to short term changes in environmental factors. This was supported by the multiple linear regression and partial correlation analyses. Significant covariation of moisture content and nematode numbers with both FDA- and AO-stained bacterial numbers in the A_{01}/A_{02}-layer was obtained (paper IV).

The FDA-stained part of the bacterial populations seemed to be more sensitive to changes in environmental conditions compared to the total bacterial populations. This was indicated by significant correlations and linear

regressions (log-transformed data) between FDA-stained bacterial numbers and accumulated precipitation for seven days before sampling date (paper IV). This was not the case with AO-stained bacterial numbers.

Soil temperature did not seem to influence the bacterial numbers to any great extent. In the same way the inorganic nitrogen content, mostly NH_4^+-N (Popovic 1976,1978 Lindberg and Popovic 1978, 1980) did not seem to be an important factor in regulating the bacterial numbers at the clear-cut area. The high concentration of inorganic N built up at Ih0 (81 µg and 71 µg inorganic N g^{-1}dw in the treatments with and without cutting residues respectively, compared to the reference stand which had 3.5 µg inorganic N g^{-1}dw) indicates bacterial activity was not limited by N deficit. This is consistent with other findings (Foster et al. 1980). In fact, Bååth et al. (1981) and Söderström et al. (submitted) showed that nitrogen fertilization had a detrimental effect on soil respiration and amounts of microorganisms in coniferous forest soils. Thus the increase in inorganic N at Ih0 could have had an inhibiting effect on the bacteria and may have contributed to the decrease in biomass after the second year. However, this was not confirmed, since no correlation between bacterial numbers and inorganic N content could be found.

LABORATORY STUDIES

The relationship between the bacteria and the bacterial feeding nematodes was studied in more detail in laboratory

experiments (paper V). A sterile soil mixture was divided into two series of bottles. The bottles in one series were inoculated with bacteria and nematodes (Acrobeloides nanus), those in the other series with bacteria alone. In a time sequence the AO- and FDA-stained bacteria were counted. Further, the nematodes were extracted with a wet funnel method and counted. Respiration rates were estimated as CO_2-evolution with a gas chromatographic technique.

The nematodes strongly affected the bacterial populations. During the period of rapid proliferation of the nematodes the bacterial growth and activity were stimulated by the presence of nematodes. This was shown by increased FDA-stained bacterial numbers and increased respiration rate in the presence of nematodes (paper V). The stimulation may have been due to increased dispersion of the bacteria by the nematode movements (Abrams and Mitchell 1980), making new nutrient sites available for colonization. Another possible explanation could be excretion of easily available nutrients by the nematodes (Anderson et al. 1983).

During the period of very high nematode abundance but low growth rate, lower bacterial numbers (both AO- and FDA-stained ones) were found in the nematode series. The respiration rate, however, was still greater in this series. The results indicate that the predation pressure exerted by the dense nematode population later in the experiments increased in importance compared to the stimulation effects. The respiration rate per bac-

terial cell indicated, however, that the stimulatory effects were still present at the end of the experiments. The specific bacterial respiration rate was at this time about 50 % greater in the series with nematodes compared to the one without.

CONCLUSIONS

The soil bacterial community consists of physiologically very different organisms. It is thus very difficult to find a single method for quantification for example, which includes all these organisms. In the laboratory experiments (paper I) it was shown that the staining ability of FDA varied with different bacterial strains. In addition, the environmental conditions seemed to influence the staining ability. In investigations with the purpose to compare different microbiological methods for soil studies (Domsch et al. 1979, Bååth et al. manuscript), it was shown that the different methods gave different results. It is thus concluded that no method can be regarded as superior to other ones, and that the decision on which method(s) to use must be influenced by the purpose of the study.

The dependence of bacteria on soil moisture content is well established (e.g. paper IV, Campbell and Biederbeck 1976). It is, however, difficult to compare results from different studies, since moisture content (e.g. expressed as weight per unit weight of soil) does not tell so much about the water status. For such comparisons

the water potential (or water activity) would give more information. In laboratory experiments the effects of the water potential on bacterial respiration and movements in soil have been studied by Wilson and Griffin (1975) and Wong and Griffin (1976) respectively. However, for comparisons over time in a specific soil, the soil moisture content, estimated e.g. gravimetrically, is useful as assessment of the water condition.

Rainfall may result in translocation of microorganisms (Madsen and Alexander 1982) and nutrients in soil due to percolation of rainwater through the soil. This may result in increased bacterial populations. This was also the case with FDA-stained bacterial numbers but not the AO-stained ones, indicating a greater susceptibility of the FDA-stained part of the bacterial populations than of the AO-stained one to short term changes in the environment. By contrast, Clarholm and Rosswall (1980) found responses in AO-stained bacteria to rainfall. Their studies, however, were carried out during short periods of intense sampling.

Despite several laboratory investigations (e.g. Bååth et al. 1978, 1981, Coleman et al. 1978, Anderson et al. 1981, Abrams and Mitchell 1980, paper V) the interactions between soil bacteria and bacterial feeding nematodes are not fully known. This was indicated by the discrepancies in results between field investigations (paper IV) and laboratory experiments (paper V). More work is needed on this matter.

Clear-cutting of the pine forest caused drastic changes in the bacterial biomass. The significance of these changes for silvicultural practices is difficult to assess. However, decreased bacterial biomass (paper III) and fungal biomass (Bååth 1980), and increased inorganic N content in the soil indicated the inability of the microorganisms to immobilize plant nutrients and to reduce possible nutrient loss from the ecosystem during the period of bare land.

The changes in environmental factors that occur after disturbances of the ecosystem, like clear-cutting, may cause a general stress to the microorganisms. This was indicated in other investigations in addition to paper III. Acidification caused a decrease in bacterial numbers (Bååth et al. 1979, 1980). Similar results were found in fertilized coniferous forest soils (Bååth et al. 1981, Söderström et al. submitted). Continued investigations of the behaviour of the microorganisms in a changing environment will probably give us more knowledge of great value in understanding the ecology of microorganisms.

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I.

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Fluorescein diacetate as a stain of metabolically active bacteria in soil

Björn Lundgren

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Fluorescein diacetate (FDA) was applied as a vital stain to soil bacteria in pure cultures, sterilized soil, and natural soils.
The staining ability of FDA was tested on 111 isolates of soil bacteria in pure cultures in a diluted laboratory medium and in sterilized soil. Nearly 80% of the isolates were stained. In a growth experiment in sterilized soil, FDA was compared to acridine orange (AO). At a given time, when most bacteria were considered metabolically active, the number of FDA-stained bacteria exceeded that stained by AO.
The staining efficiency and the simple procedure indicate that the FDA-method is useful for estimating the number of metabolically active bacteria in soil. This was confirmed by examination of ten different soils, where the FDA-method was compared to AO-staining, fluorescein isothiocyanate (FITC)-staining, and plate counts.

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Флуоресцирующий диацетат (FDA) использован для прижизненного окрашивания почвенных бактерий в чистых культурах, стерилизованной почве и необработанной почве.
Определяли окрашивающую способность FDA на 3 изолатах почвенных бактерий в чистых культурах на разбавленной лабораторной среде или в стерилизованной почве. Покрасилось около 80% изолятов. В опытах по росту на стерилизованной почве FDA сравнивали с активном оранжевым (AO). В определенный период, когда бактерии в большинстве своем были метаболически активны, число бактерий, окрашенных FDA, было больше, чем окрашенных AO. Высокая эффективность окрашивания и простота процедуры показали, что метод FDA пригоден для оценки количества метаболически активных бактерий в почве. Это подтверждается и проверкой 10 различных почв, где метод FDA сравнивали с результатами окрашивания AO, флуоресцирующим изотиоцианатом (FIIC) и методом счета на стеклах.

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1. Introduction

During the past few years the interest in microbial biomass and activity in soil has increased, partly because of the importance of the data in integrated ecosystem studies. Consequently a number of methods have been developed to estimate bacterial biomass in soil, including microscopical counting methods. Most of these direct counting methods make use of different fluorescence staining techniques.

It has been suggested that only a minor part of the bacterial soil flora is metabolically active at a given moment (Babiuk and Paul 1970). It would thus be desirable to obtain information about the activity of the cells counted. By staining soil smears with europium chelate and fluorescent brightener (Anderson and Slinger 1975) information about the metabolic state of the microbial cells can be obtained. This technique differentiates between living (or recently dead) and dead cells, but gives no information of the activity. Mayfield (1977) presented another fluorescence staining method for the counting of viable microorganisms in soil, but since this method is very laborious it is not suitable for routine purposes.

Fluorescein diacetate (FDA) is a nonfluorescent substance which, after entrance into cells, will be hydrolysed to fluorescein by suitable enzymes (e.g. esterases), and hence the cells will glow in UV-light (Rotman and Papermaster 1966). However, when staining soil bacteria with FDA, Babiuk and Paul (1970) did not consider the method useful on a routine basis. Paton and Jones (1975), on the other hand, described a technique using FDA as a stain for bacteria and yeasts in fluids. The use of this stain on pure cultures of bacteria and fungi was reported by Ziegler et al. (1975) and Söderström (1977). The latter author also described a technique for staining fungi in soil preparations.

Since the method of Paton and Jones (1975) was not suitable for bacteria in soil preparations, an improved FDA-method was needed. Such a technique is presented in this paper. The staining efficiency of FDA was investigated and comparisons with other counting methods were also made.

2. Materials and methods

2.1. Soil dispersion

The soils used are listed in Tab. 1. An appropriate amount of soil was homogenized in 250 ml of a filtered (Millipore 0.45 μm) solution of 0.2% (w/v) sodium hexametaphosphate (Calgon, BDH Chemicals Ltd) and 0.1% (w/v) Proteose Peptone (Oxoid), below abbreviated as C-P-solution. The dispersion was made in a MSE Atomix blender, run at top speed for 2 min. The soil suspension was then transferred to a 500 ml graduated cylinder and allowed to settle for one minute before subsamples were taken out.

Tab. 1. Soil characteristics.

No.	Soil	pH*	% LOI*
I.	Garden soil	7.3	15.0
II.	Garden soil	7.0	7.9
III.	Pasture, sandy soil	7.6	3.9
IV.	Sand dune soil	5.7	0.7
V.	Mull (A_1), *Fagus sylvatica* L. forest	4.0	17.0
VI.	Humus (A_{01}/A_{02}), *Pinus sylvestris* L. forest	4.1	92.3
VII.	Mineral soil (B), same site as no VI	4.8	2.6
VIII.	Humus (A_{01}/A_{02}), *Pinus sylvestris* L. forest	3.9	91.1
IX.	Humus (A_{01}/A_{02}), *Picea abies* (L.) Karst. forest	3.5	56.7
X.	Mineral soil (B), same site as no. IX	3.7	6.2

* = pH determined in 1:2 w/v soil : H_2O ratio, % LOI = % loss on ignition of dry soil (850°C, 4 h).

2.2. Staining procedure

The procedure described by Söderström (1977) was followed, with the exception of the buffer and membrane filter used. A subsample from the dispersed soil suspension was diluted in filtered (Millipore 0.22 μm) 0.50 M phosphate buffer, pH 8.5, which was then stained for 3 min with 10 $\mu g \cdot ml^{-1}$ fluorescein diacetate (FDA, Koch-Light Laboratories Ltd, Colnbrook, England). The suspension was then filtered through a membrane filter (Millipore 0.22 μm) dyed with Dylon No. 8 (Ebony Black, Dylon International Ltd, London, England) to reduce the autofluorescence of the filter (Jones and Simon 1975). The filter was then embedded in nonfluorescent immersion oil (Cargille Type B) on a glass slide.

The preparation was examined under a Reichert Zetopan microscope (magnification 800×, 100× oil immersion objective, NA = 1.25) with incident UV-light from a Binolux light source (HBO 200 Hg lamp). A 3 mm BG 12 was used as a primary filter and 1 mm GG 9 + 2 mm OG 515 as the secondary filter.

A number of modifications except for the ones described above were also examined. Prolonged staining time (6 or 10 min) increased the background fluorescence without increasing bacterial counts, while shorter staining time (1.5 min) gave lower bacterial counts. Different buffers, phosphate buffers of different concentrations 0.06–0.50 M, and pH-values 5.5–8.5, and Tris- and Veronal buffers, were tested. Counterstaining with 0.125% (w/v) Rhodamine B (BDH Chemicals Ltd) for 2 min, and the addition of sodium acetate (0.06 M) just before FDA-staining were also tested (Portno and Molzahn 1977). None of these modifications im-

proved the results, and thus the preparation scheme already described was used in all further experiments.

2.3. Staining of pure cultures

Pure cultures, isolated from spread plates of soils No. I, V, and VII (Tab. 1), were tested for ability to be stained by FDA. The spread plates used for soils No. I and VII were 0.3% TSA-plates (Tryptic Soy Broth, Difco, +1.5% Bacto Agar, Difco, Martin 1970), supplemented with 70 ppm cycloheximide (Sigma), and for soil No. V soil extract agar plates (Kauri 1978) were used.

The isolates were incubated in 0.5% TSB (Tryptic Soy Broth) in tubes on a rotary shaker at 22°C. They were sampled during the logarithmic growth phase (determined by nephelometry) and stained with FDA.

Those isolates that did not show fluorescence after FDA-staining were harvested by centrifugation and washed in sterile tap water. They were then inoculated into heat sterilized garden soil. Sampling for FDA-staining was then made after 24 h at 22°C and the cultures were reinoculated after 48 h into new soil cultures. This was repeated up to five times.

When positive results were obtained in soil cultures, the bacteria were inoculated into laboratory medium to check for purity, as well as into 0.5% TSB-tubes to determine FDA staining ability. Isolates showing negative results in the last soil culture were checked for viability on 0.5% TSA-plates.

2.4. Growth experiments in soil

To investigate the staining efficiency of FDA in mixed populations a growth experiment was designed. Humus (soil No. VIII, Tab. 1) and mineral soil (B horizon at the same site) were carefully mixed in a 1:8 ratio (dry weight) and divided into 30 erlenmeyer flasks (18.7 g dry soil in each). After moistening, the flasks were autoclaved at 120°C for one hour on three subsequent days. The pH value of the soil mixture after sterilisation was 4.2 (soil : H_2O 1:2, w/v). The moisture content was adjusted to 80% of water holding capacity. The flasks were then inoculated with 0.50 g fresh mineral soil per flask and incubated at 20°C. During the incubation time of 20 d the moisture content dropped to 70% of water holding capacity.

Three parallel flasks were examined for bacterial number every 24 h during the first 6 d, and thereafter at longer intervals. The soil in each of the flasks was homogenized separately in 250 ml C-P-solution, diluted and stained with FDA as described above.

To obtain total counts the homogenized suspension was diluted in C-P-solution and stained with acridine orange (AO, BDH Chemicals Ltd) at a concentration of 67 μg ml^{-1} for 5 min. It was then filtered through a dyed filter (Millipore 0.22 μm), and examined in the same way as the FDA preparation.

The experiment described above was repeated twice, once with the same humus : mineral soil ratio of 1:8, and once with a ratio of 1:2.

2.5. Comparisons between methods

To evaluate the FDA-staining technique against other methods of bacterial counting, soil suspensions were made from ten different soils (Tab. 1). Samples were taken out for preparation of FDA-stained suspensions, acridine orange stained soil smears (Trolldenier 1972), fluorescein isothiocyanate (FITC)-stained smears (Babiuk and Paul 1970), and plate counts on 0.3% TSA (see above). Three replicate preparations were made for the direct counting methods. For the plate counts the soil suspensions were diluted in 0.3% TSB and spread on five replicate plates. After incubation at 20°C for two weeks the plates were examined.

The FDA-staining technique described in this paper was also compared to that of Paton and Jones (1975). In the latter method the soil suspension was filtered on a black membrane filter, which was then stained in a 0.01% FDA-solution in buffered saline (pH 7.5) for 5 min before mounting and examined under the microscope. Three replicate preparations were made for each technique from a common soil suspension (soil No. III).

3. Results

3.1. Staining of pure cultures

Of the 111 tested isolates, grown in TSB, 74 showed fluorescence after FDA-staining during the logarithmic growth phase. Under the growth conditions used the intensity ranged from rather weak to very strong. In all cases it was easily separated from that which could be regarded as autofluorescence of i.e. soil debris in soil preparations. A few isolates, positive in TSB, were also inoculated into soil culture. All of these showed positive results.

The 37 isolates showing negative results in TSB were inoculated into sterile soil. After the first inoculation 5 isolates showed positive results. After the third reinoculation an additional three, and after the fifth altogether 13 soil cultures showed positive results. Seven isolates did not grow in the soil.

Totally, 87 (78%) isolates glowed after FDA-staining in either TSB or soil, while 17 (15%) growing isolates did not glow. No difference could be detected between isolates from different soils or between those isolated on different media. Both Gram positive and Gram negative bacteria were stained.

To investigate the staining ability of FDA in different growth stages, FDA-preparations were made using a *Bacillus* strain, isolated from soil No. VIII and grown in Tryptic Soy Broth. The bacterium lost the staining ability completely after heat-killing (10 min in a boiling water bath), while the growing culture showed strong fluorescence. During the sporulation process, a dark

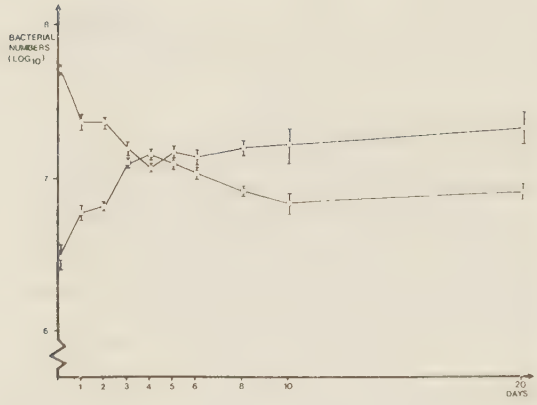

Fig. 1. The numbers of bacteria, stained with acridine orange (○) and fluorescein diacetate (□) respectively, in a growth experiment. A sterilized humus : mineral soil mixture (1:8 ratio) was inoculated with fresh mineral soil. Bars indicate the 95% confidence limits.

spot, increasing in size, was observed in the glowing bacterial cell. When sporulation was completed, almost all the fluorescence was lost. The occurrence of endospores was confirmed by staining with malacite green.

3.2. Growth experiments in soil

The development of FDA-stained and AO-stained bacterial populations was followed in a sterile soil inoculated with an unknown mixed population. The results in one experiment with a humus : mineral soil ratio of 1:8 are shown in Fig. 1. The initially high value of AO-stained bacteria was due to dead cells remaining in the sterilized soil, since uninoculated soil gave the same amount of cells. The low initial number of FDA-stained bacteria (6% of AO-stained ones) all emerged from the inoculum.

During the first few days (see Fig. 1) the FDA-value increased rapidly, while the AO-value decreased. On the fourth day the FDA-count reached its maximum value, and the AO-count its minimum value. At this time the number of FDA-stained bacteria was significantly higher ($p < 0.05$, Student's t-test) than the AO-count. After the fourth day the FDA-value dropped and the AO-value increased slightly. Then the system seemed to stabilize at about the 10th day, when the FDA-count was about 40% of the AO-count. With both staining methods a great variation in size and morphology of bacterial cells was observed during the experiment.

A second experiment with the same humus : mineral soil ratio gave similar results. In an experiment with a 1:2 humus : mineral soil ratio the development of the populations was much faster. After two days the number of FDA-stained bacteria was as high as the initial number of AO-stained ones. Already in the third day the FDA-value had reached a maximum ($\log_{10} = 9.55 \cdot gdw^{-1}$) which was much higher than in the previous experiments. Due to the fast development in this experiment a decrease in AO-count was not detected. Instead a lag-period of one to two days preceded the increase. This system stabilized after about 5 d. Again the FDA-value was about 40% of the AO-value.

3.3. Comparisons between methods

The FDA-staining method described by Paton and Jones (1975) gave a stronger background fluorescence resulting in a weaker contrast in the preparations as compared to the method described in this paper. In the soil studied, their method gave 7.97 bacterial cells per field of view (standard error 0.88), while the method reported in this paper gave 21.8 cells per field of view (s.e. 0.77). The difference was significant ($p < 0.01$, Student's t-test).

The FDA-method was compared with AO- and FITC-staining techniques and plate counts in 10 differ-

Tab. 2. Comparison between direct counts and plate counts in ten different soils. Mean of three estimations ± SE.

Soil no.	FDA* ($\cdot 10^{-9} \cdot gdw^{-1}$)	AO* ($\cdot 10^{-9} \cdot gdw^{-1}$)	FITC* ($\cdot 10^{-9} \cdot gdw^{-1}$)	Plate counts* ($\cdot 10^{-6} \cdot gdw^{-1}$)
I.**	0.85±0.03	2.65±0.06	0.99±0.01	335 ±20
II.	1.38±0.04	4.23±0.20	2.01±0.10	174 ±16
III.	0.08±0.01	0.26±0.03	0.17±0.02	4.90±0.48
IV.	0.04±0.01	0.11±0.01	0.05±0.01	0.12±0.01
V.	2.34±0.06	6.16±0.13	1.59±0.16	9.89±1.16
VI.	5.92±0.31	13.9 ±1.5	4.75±0.18	15.8 ±1.1
VII.	0.28±0.01	0.76±0.05	0.19±0.02	0.13±0.03
VIII.***	0.14±0.02	7.18±0.42	3.42±0.28	3.68±0.51
IX.	1.65±0.02	6.09±0.78	2.28±0.17	4.77±0.29
X.	0.39±0.03	0.47±0.04	0.75±0.09	2.92±0.39

*; AO = Acridine orange stained, FITC = Fluorescein isothiocyanate stained, FDA = Fluorescein diacetate stained bacterial numbers. Plate counts on 0.3% Tryptic Soy Broth + 1.5% Bacto Agar + 70 ppm cycloheximide.
**; Air dried, rewetted soil, incubated for 3 d at room temperature.
***; Soil frozen at –20°C for three months.

ent soils. The results are presented in Tab. 2. The FDA-values ranged between 2 and 83% of the AO-values and between 4 and 147% of the FITC-values. The plate counts were in the order of 10 to 1000 times lower than the direct counts except for soil No. 1.

Comparisons between the FITC- and AO-staining techniques, which are both supposed to give total counts, show that the FITC-values were 35 to 75% lower than the AO-values except in soil No. X where the FITC-value was higher than the AO-value.

The FDA-preparations were of a good quality in all soils tested, although the contrast was somewhat better with humus soils. This also holds for the AO-preparations. The FITC-preparations, however, showed a better quality with the garden soils.

4. Discussion

Ziegler et al. (1975) and Paton and Jones (1975) did not report any difficulties in the staining of bacteria with FDA. In the study reported here the majority of the bacterial isolates were stained in pure cultures. However, 33% of the isolated soil bacteria showed a negative response to FDA-staining, when grown in laboratory medium. Since FDA is hydrolysed rather unspecifically (Rotman and Papermaster 1966) the reason is probably not as simple as a lack of suitable enzymes. Instead a change in membrane properties might occur, resulting e.g. in leakage of fluorescein out of the cells or decreased upptake of FDA. Changes in growth medium may cause changes in the physiology of the bacterial cells, including changed enzyme-substrate affinities (Tempest and Neijssel 1978). Thus the negative results obtained in TSB do not necessarily mean that these bacteria are not stained in soil. In fact, when grown in sterilized soil, nearly half of them glowed after FDA-staining.

The staining behaviour of a *Bacillus* strain during sporulation and the lack of fluorescence when heat-killed cells were stained, support the conclusion of Ziegler et al. (1975) that FDA stains metabolically active organisms only. The results of the growth experiments in soil (Fig. 1) also indirectly support this conclusion. The growth curve obtained with the FDA-method is in agreement with earlier findings with cultural methods in autoclaved and gamma irradiated soils (Salonius et al. 1967) and in fumigated soil (Ridge 1976). The AO-curve, however, does not fit into this pattern for living bacteria. It is interesting to note that the FDA-stained cells on the fourth day, when they were most numerous, exceeded the AO-count. This would mean that in a situation where most soil bacteria are in a metabolically active state, the FDA-method is more efficient than the AO-staining method, which is considered to give total counts (Trolldenier 1972). The higher content of organic material, when a humus : mineral soil ratio of 1:2 was used, resulted in a faster growth rate of the bacteria. This also supports the conclusion above concerning the selective feature of FDA.

Percentages like those given above (FDA- of AO-counts) must, however, not be regarded as true estimates of the active part of the bacterial population, since the counts obtained are highly dependent on the preparation procedure used. This is emphasized by the difference in results between the technique described here and the one of Paton and Jones (1975).

The comparisons between methods show that FDA is useful as a stain in highly different soils. The FDA-counts varied between 2 and 83% of the total counts (AO-method, Tab. 2). The lowest percentage was obtained in frozen humus, where no or very low activity was expected after three months storage at –20°C. Except for this soil the lowest percentage was 27 (soil No. IX). The absolute values were lowest in the sand dune and sandy pasture. This is to be expected since they had low contents of organic matter (LOI, Tab. 1) and were very dry upon sampling.

The FDA-method has for some time been used for monthly estimations of soil bacteria in a podzolic pine forest soil in Central Sweden. In the A_{01}/A_{02}-horizon the mean number of FDA-active bacteria was about $7 \cdot 10^9$ cells gdw^{-1}, and was 30–130% of the total number (AO-staining method by Trolldenier 1972). This investigation will be published elsewhere. Mayfield (1977) reported that 8% of the cells multiplied in his investigation. However, as Mayfield stated, addition of nutrient medium, which is a part of his method, gives a selective effect when incubating the preparations. Cells stained with FDA indicate an activity at the actual sampling time in the soil and they are not exposed to selective effects of the same kind as a nutrient addition would give.

Some other advantages of the FDA-staining method are also worth mentioning. As humus and mineral particles are not stained by FDA, they do not show any (or very weak) fluorescence, and hence the bacteria are more easily distinguishable in the FDA- than in the AO-preparation. The preparation procedure is rapid and easy, allowing a sample to be dispersed, prepared and examined in 20 min or less. The main advantage of the method is however, that in contrast to AO, FDA appears to selectively stain metabolically active bacteria. As there is no absolute proof that FDA stains all active bacteria, the method is most useful in comparative studies, e.g. comparison of different treatments of the soil or in a study of fluctuations of the bacterial population with time.

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Added after publication:

ERRATA:

Page 19, line 8: "1970" should be "1975".

Page 22, add:

Martin, J.K. 1975. Comparison of agar media for counts of viable soil bacteria. Soil Biol. Biochem. 7: 401-402.

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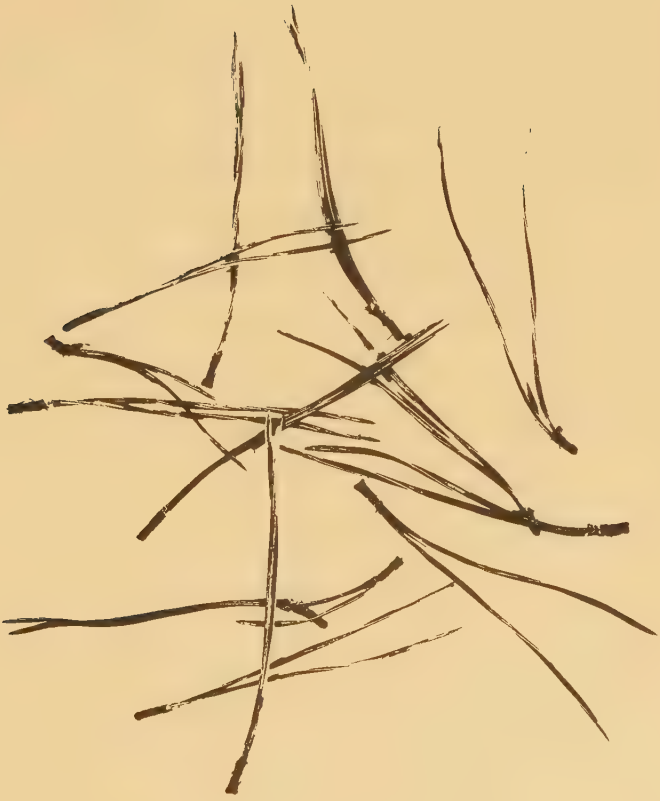

EFFECTS OF SIZE CLASSIFICATION ON MICROSCOPICALLY ESTIMATED BIOVOLUMES OF SOIL BACTERIA

by

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Methods for estimating microbial biomass were recently reviewed by Jenkinson and Ladd (1981). For estimates of soil bacterial biomass direct counting methods are extensively used. Of these, fluorescence microscopical techniques are preferred today (e.g. Babiuk and Paul 1970, Trolldenier 1972, Lundgren 1981). To obtain biomass values, the biovolumes are first estimated, and then multiplied by a volume-weight conversion factor. The biovolume is commonly calculated by multiplying the bacterial number by the average cell volume. Since measuring individual cells is very time consuming, the average cell volume is most often estimated by a size classification method (e.g. Jenkinson et al. 1976, Clarholm and Rosswall 1980). In the present investigation measurements of individual cell volumes are compared to volumes estimated with size classification of the cells. The effect of different class limits in the classification procedure was also studied.

Samples were taken on four occasions, April, May, June and November 1982, in a podzolic soil under a mixed forest of Scots pine (Pinus sylvestris L.) and Norwegian

spruce (Picea abies (L.) Karst.) in southern Sweden. Five replicate samples of the organic soil layer (A_{01}/ A_{02}) were taken at each occasion and were processed the same day.

For bacterial estimates soil smears were stained with acridine orange (AO) (Trolldenier 1982, as modified by Clarholm and Rosswall 1980). The microscope used was a Reichert Zetopan with incident UV-light (HBO 200 Hg lamp) and 800x magnification. Size classifications of bacteria were made using both the classes presented by Clarholm and Rosswall (1980) and the ones used by Lundgren (1982), which are presented in Table 1. Five fields of view per sample were analysed and 20-40 bacteria in each were classified. In the November sampling, the average cell volumes obtained by measuring individual cells were compared with those of the size classification according to Lundgren (1982).

Tab. 1

The average cell volume obtained was 0.13 ± 0.03 (standard error) μm^3 for the classification according to Lundgren (1982), and 0.54 ± 0.06 μm^3 for that of Clarholm and Rosswall (1980) on the first three sampling occasions. In nested ANOVAs (Sokal and Rohlf 1981) no statistically significant difference in average volume between samples or sampling dates was found. Most of the variation in average cell volume was due to variation between fields of view, which was included in the error term of the statistical analysis. To find differences in biomass between samples or sampling dates, the numbers of fields of view or the numbers

of bacteria per field of view would have to be increased considerably. However, the latter is not recommended, since increasing the amount of soil on the soil preparations would cause masking of bacteria by the soil particles (Faegri et al. 1977).

Ninty-eight per cent of the bacterial cells fell into the two smallest size classes in both classification systems, i.e. classes no. I and II of Lundgren (1982) and I and IV of Clarholm and Rosswall (1980). The considerable difference in average cell volumes between these classification systems were thus due to differences in nominal class volumes (caused by differences in class limits) in the two smallest classes.

Jenkinson et al. (1976) and Chuang and Ko (1981) found an inverse linear relationship between numbers and sizes of microorganisms on a logarithmic scale. The biased distribution of bacteria in the size classes (see above and the cell numbers in Fig. 1) indicate a similar relationship in the bacterial populations of the soil used in this study. Because of this distribution the average cell volumes were overestimated by using size classification and nominal class volumes. The total biovolume of each size class, estimated both by individually measured cells and nominal class volumes, is shown in Fig. 1. When measuring the bacteria individually (totally 250 cells in five samples from November), an average cell volume of 0.12 $\pm$ 0.01 (standard error) μm^3 was obtained, while classification according to Lundgren (1982) of the same cells resulted

Fig. 1.

in an average cell volume of 0.15 ± 0.01 μm^3. The classification thus resulted in a biovolume 25 % greater than measurements of individual cells. According to the results of the first three samplings, the classification system of Clarholm and Rosswall (1980) overestimated the average cell volume by about 5 times. Obviously, a correction factor is necessary when size classification is used.

As mentioned above the vast majority of bacteria was smaller than 1.0 µm, and about 55 % were smaller than 0.5 µm (Fig. 1). Jenkinson et al. (1976) and Balkwill et al. (1975) also found that the majority of bacteria was smaller than 0.5 µm in preparations for light microscope and transmission electron microscope (TEM). It would thus be advantageous to divide the bacteria smaller than 1 µm into more detailed size classes to obtain better precision in estimates of average cell volume. However, except for TEM, this is not meaningful, because of the optical limits of the conventional light or fluorescence microscope.

This study has shown that when size classification is used, it is extremely important to select proper class limits, which should be compared to direct measurements of cells in a pilot study. Furthermore, for biovolume estimates it is desirable that the number of fields of view per sample (or preparation) is as high as possible. However, this is dependent on the precision wanted and the resources available.

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Table 1. Class limits and nominal class volumes for size classification of soil bacteria, according to Lundgren (1982) and Table 2 in Clarholm and Rosswall (1980).

Class no.	limits (µm)	nominal volume (μm^3)
Lundgren (1982):		
I	$\leq 0.5 \times \leq 0.5$	0.03
II	~0.5 x (0.5-1.0)	0.22
III	~0.5 x (1.0-2.0)	0.34
IV	>0.5 x >2.0	0.75
V	$\geq 1.0 \times \geq 1.0$	1.77
Clarholm and Rosswall (1980):		
Spheres		
I	<1.2	0.3
II	1.2 - 1.6	1.4
III	>1.6	4.2
Rods		
IV	<2.0 x <1.0	0.8
V	2.0-4.0 x 1.0-1.4	2.4
VI	>4.0 x >1.4	10.0

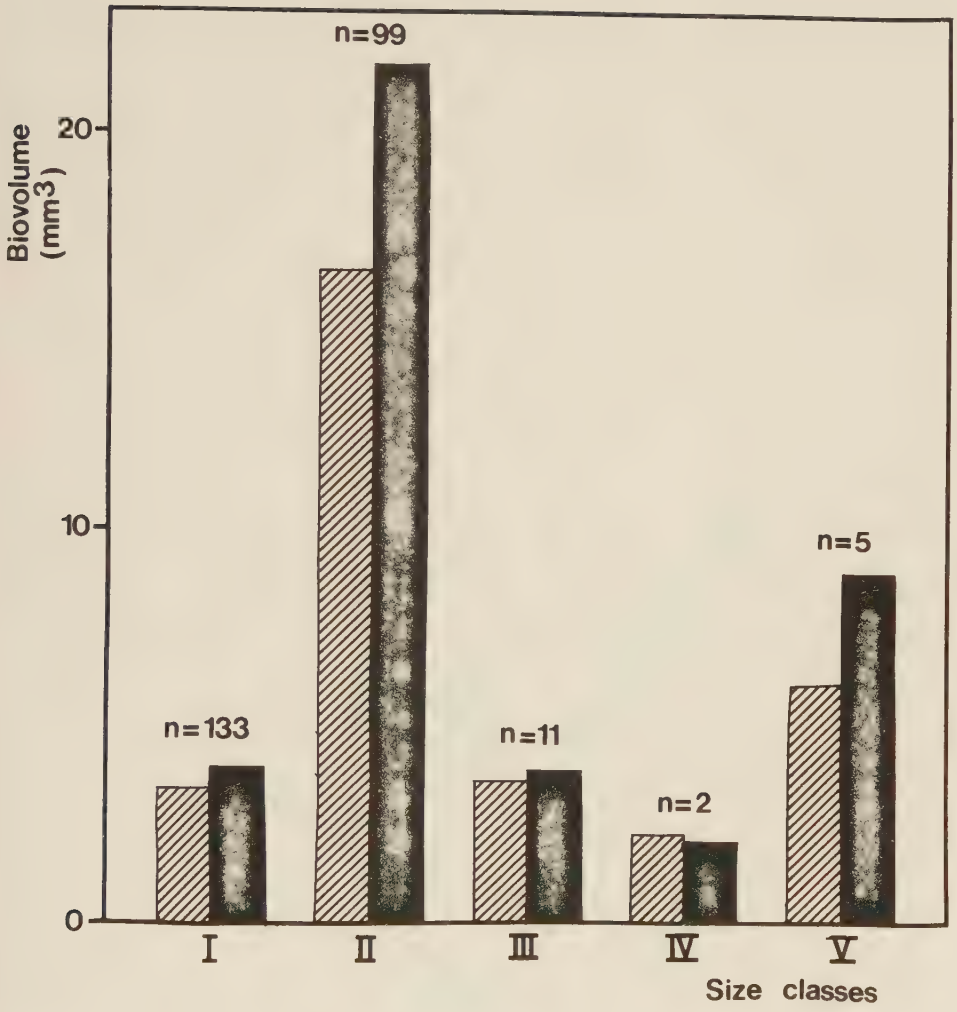

Fig. 1. Comparison of biovolume in each size class using the classes of Lundgren (1982) from the November sampling. The striped bars indicate the volume of individually measured cells falling into each class, and the black bars indicate the volume estimated with nominal volumes multiplied by the numbers of bacteria in each size class. n = total number of bacteria in each size class.

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BACTERIA IN A PINE FOREST SOIL AS AFFECTED BY CLEAR-CUTTING

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Summary—Clear-cutting a Scots pine stand resulted in an initial increase in bacterial biomass determined by microscopic counts after staining with both acridine orange (AO) and fluorescein diacetate (FDA). During the third year after clear-cutting and onwards, bacterial biomass decreased compared to a reference stand. Results from a *ca.* 10-yr old clear-cut area indicated long-lasting detrimental effects on the size of bacterial populations. Plots in the clear-cut area which received slash consistently contained greater amounts of bacteria than did plots with slash removed.

The FDA staining was used for the first time in long-term field measurements and gave similar results to those obtained with AO.

INTRODUCTION

The boreal coniferous forest ecosystem normally has a very tight circulation of plant nutrients. Nutrients mineralized by soil microorganisms are immediately absorbed by plant roots, and nutrient losses from the system are small. However, undesirable nutrient losses might occur when the closed nutrient cycle is broken by disturbance of the ecosystem. Clear-cutting of the forest is such a disturbance. Wiklander (1981) has shown that one result of clear-cutting is increased leaching of nitrogen with subsequent loss of this nutrient from the system.

Clear-cutting is virtually the only way of harvesting timber in large areas of the boreal region. Considering this fact and the potential risk of losing large amounts of plant nutrients by this operation, studies of the effects of clear-cutting on mineralizing organisms are surprisingly sparse. Effects of clear-cutting on fungi was studied by Bååth (1980, 1981; further references in these papers). Laudelout *et al.* (1978) showed increased viable bacterial counts in a spruce forest soil in Belgium 1 yr after clear-cutting. Grunda (1964) also showed increased bacterial plate counts in clear-cut areas in Czechoslovakia. In northern Finland Sundman *et al.* (1978) found greater bacterial populations (viable counts) in up to 7-yr old cuttings compared to a reference spruce stand.

Studies on microorganisms in manipulated systems are of interest not only in general ecology and forestry, but also in soil microbiology, since investigations of populations in disturbed situations help us to understand and interpret results from the natural, undisturbed system. I report an investigation of effects of clear-cutting on soil bacteria enumerated by direct counting methods during a period of 5 yr. Since removal of slash is of interest in forestry, e.g. for energy production, investigation of effects on bacterial biomass of no slash compared to double amounts of slash on the ground was included.

MATERIALS AND METHODS

Sites

The research area was the main site of the Swedish Coniferous Forest Project at Ivantjärnsheden, near Jädraås in the province of Gästrikland (60°49′N, 16°30′E). Annual mean temperature is 3.8°C and annual mean precipitation 607 mm (measured at Åmotsbruk, 15 km N of Ivantjärnsheden, by the Swedish Meteorological and Hydrological Institute). The ground is covered with snow for approximately 5 months (December–April).

The soil is an iron podzol on glacifluvial sandy sediment under a Scots pine forest (*Pinus sylvestris* L.) (Table 1). The ground vegetation is of dry dwarf shrub type, dominated by *Calluna vulgaris* (L.) Hull, *Vaccinium vitis-idaea* L. in the field layer, and lichens (*Cladonia* spp) and feather mosses (e.g. *Hylocomium* sp.) in the bottom layer.

Clear-cutting was done in March–April 1976 at the research site designated Ih 0. The site was divided into 12 plots (25 × 25 m). Double the normal amount of slash ($36 \pm 4.5 \times 10^3$ kg dw ha^{-1}) was left on the ground after cutting in six of the plots, and in the other six slash was removed. Another site, which was clear-cut in 1967 (Ih 1), was used for additional comparative analyses. An about 120-yr old mature pine stand (Ih VA), was used as a control site. The sites are described in detail by Axelsson and Bråkenhielm (1980). Soil bacteria have earlier been investigated at Ih VA by Clarholm and Rosswall (1980), and soil fungal biomass were investigated at all three sites by Bååth (1980), and at Ih VA by Söderström (1979).

Sampling

At the clear-cut area (Ih 0) samples were taken about monthly during the growing season, May–October/November, three successive years after cutting (1976–78). At the reference stand (Ih VA) monthly samples were taken during the growing

Table 1. Mean values of loss on ignition and pH values of soil horizons at Ih 0 and Ih VA

Soil horizon	Site	Loss on ignition (% of dry soil)	pH* (H_2O)
A_{01}/A_{02}	Ih 0 (+ slash)	79.8	4.0–4.4
	Ih 0 (− slash)	79.0	3.9–4.4
	Ih VA	82.5	3.9–4.2
A_2	Ih 0 (+ slash)	2.7	4.3–5.0†
	Ih 0 (− slash)	2.4	4.4–5.2†
	Ih VA	3.8	4.5–5.0†
B	Ih 0 (+ slash)	5.6	ND
	Ih 0 (− slash)	5.0	ND
	Ih VA	3.9	ND

* Lindberg and Popovic, unpublished results.
† Mineral soil 0–10 cm.

seasons in 1977 and 1978, but only once in 1976 (September). The old clear-cut area (Ih 1) was sampled in September 1977 and 1978. In September 1979 and 1980 additional samples were taken at all four sites.

At all sites six soil cores (6 cm dia) were taken on each occasion. Cores were transported to the laboratory in plastic bags at 5–10°C and processed within 24 h after sampling.

Analyses

Soil cores were divided into the humus layer (A_{01}/A_{02}), elluvial mineral soil layer (A_2, *ca.* 5 cm of the mineral soil), and illuvial layer (B, *ca.* 5–15 cm of the mineral soil). On all sampling occasions in 1976 and 1977 and once for each treatment at Ih 0 and Ih VA in 1978, the A_{01}/A_{02} layers of cores were analysed separately. In the mineral soil layers and on all other occasions in the A_{01}/A_{02} layer, the 6 samples were pooled.

Fluorescence microscopy was used to obtain bacterial numbers. Acridine orange (AO) staining of soil smears (Trolldenier, 1972; modified according to Clarholm and Rosswall, 1980) was used for total counts. Soil suspensions were stained with fluorescein diacetate (FDA) to estimate the number of metabolically-active bacteria (Lundgren, 1981) in 1977 and 1978. FDA-stained bacteria were usually counted in pooled samples. On three occasions, once for each treatment at Ih 0 and Ih VA in 1978, the A_{01}/A_{02} layers of cores were analysed separately for FDA-stained bacteria.

Bacterial dry weights were estimated after size classification of cells. A density of 1.3 g cm^{-3} and a dry weight of 20% of wet weight (Faegri *et al.*, 1977) was used in the calculations. The bacterial biomasses are presented on a m^2-basis.

Soil moisture content was estimated gravimetrically (drying at 105°C overnight), and organic matter content as loss on ignition (850°C for 4 h, Table 1).

Two-factor ANOVA was used for statistical analyses, with treatment (Ih 0 with and without slash and Ih VA) and years as the two factors. Analyses were made on log transformed data. Standard error was calculated as the antilogarithm of the square root of the mean square in the error term of the ANOVA (Snedecor and Cochran, 1967, p. 330). It is presented as per cent of the annual mean for each treatment. Partial correlation coefficients were calculated taking into account AO-stained or FDA-stained bacterial numbers, moisture content, and loss on ignition. The calculations were made separately for AO-stained and FDA-stained bacteria. The two treatments at Ih 0 were treated separately when calculations were based on separate soil cores, and together when pooled samples were used. Log transformed data were used in the analyses.

RESULTS

The range for numbers of AO-stained bacteria in the A_{01}/A_{02} horizon was 5.8–63.3 × 10^9 gdw^{-1} (of soil) and for FDA-stained bacteria 2.6–20.0 × 10^9 gdw^{-1}. In the A_2 horizon bacterial numbers ranged from 0.66 to 2.17 × 10^9 and from 0.20 to 1.21 × 10^9, and in the B horizon 0.60–2.60 × 10^9 and 0.15–0.75 × 10^9 gdw^{-1}, for AO-stained and FDA-stained bacteria, respectively. Results are presented and discussed below as biomasses (dry weight) of AO-stained and FDA-stained bacteria.

Differences between treatments were highly significant, and there were also significant differences between the 2 yr, 1977 and 1978 (Table 2). Significant interaction terms were found only in the A_{01}/A_{02} horizon. AO and FDA stains gave about the same results with a tendency towards higher *F*-values in the first factor (treatment) for the FDA-stained bacteria. An

Table 2. *F*-values and levels of significance for 2-factor ANOVAs. Treatments (Ih 0 with slash, Ih 0 without slash, Ih VA) was one factor and years (1977, 1978) was the second factor. Analyses were made with log-transformed bacterial biomass per m^2

		A_{01}/A_{02}	A_2	B	$A_{01}/A_{02} + A_2 + B$
AO-stained bacteria	Treatment (T)	29.6***	121.5***	8.4**	35.8***
	Year (Y)	5.1*	5.5**	11.2**	16.6***
	Interaction (T × Y)	9.3**	2.8 NS	0.4 NS	3.4*
FDA-stained bacteria	(T)	30.6***	98.8***	19.8***	56.4***
	(Y)	14.9***	4.3*	8.6**	16.9***
	(T × Y)	5.9*	2.0 NS	0.4 NS	2.1 NS

*** = Significant at the 99.9% level.
** = Significant at the 99% level.
* = Significant at the 95% level.
NS = Not significant.

additional ANOVA calculation was made for AO-stained bacteria which included data from 1976. Ih VA was excluded from this analysis since there was only one sampling occasion from 1976. Results were similar to those presented in Table 2, except that for mineral soil layers no significant difference was found between the treatments. Ih 1 was not included in these analyses.

Effects of clear-cutting

Annual mean values for AO-stained and FDA-stained bacterial biomass per m^2 of the different sites and horizons are presented in Fig. 1.

At the clear-cut site (Ih 0) AO-stained biomass increased from the first to the second year in the A_{01}/A_{02} horizon. During the second year both AO-stained and FDA-stained bacteria were found in much greater biomass in the clear-cut area compared to the reference stand (Ih VA). During the third year values dropped again at Ih 0; for plots without slash on the ground biomass decreased even below that at Ih VA. At Ih 1 bacterial biomass was comparably small both years (1977 and 1978).

In the A_2 horizon bacterial biomass was greatest during the second year at Ih 0. The large difference between the reference stand and Ih 0 is difficult to interpret, however, due to differences in procedures used to estimate the soil weight m^{-2} in this horizon. Ih 0 probably had greater biomass than Ih VA, since Ih 0 had an average of 1.33×10^9 and Ih VA 1.07×10^9 AO-stained bacterial cells gdw^{-1} during the period of investigation, and corresponding values for FDA-stained bacteria were 0.68×10^9 at Ih 0 and 0.39×10^9 bacteria gdw^{-1} at Ih VA.

Bacterial biomass at Ih 0 was also greatest during the second year in the B-horizon. By the third year there was a marked decrease in biomass, while values at Ih VA were almost constant.

Ih 1 had about the same biomass of AO-stained bacteria as Ih 0 in mineral soil layers in September 1977 and 1978. FDA-stained biomass values were lower at Ih 1 than Ih 0, however.

The pattern followed by bacterial biomass in the whole profile ($A_{01}/A_{02} + A_2$ and B-horizons) was largely determined by the A_{01}/A_{02} layer. At Ih 0 there was an increase between the first and second years and a decrease between the second and the third years. At Ih VA values were constant during 1977 and 1978.

Relative biomass of bacteria at Ih 0 and Ih 1, expressed as differences between these sites and Ih VA, are shown in Fig. 2. These values are based on dry weight of soil. The effect of different soil weights m^{-2} is thus eliminated which makes detection of differences due to other soil factors e.g. moisture possible. For convenience values of September 1976 at Ih VA of AO-stained bacteria are compared to the values of September at Ih 0 this year. Also, all values of Ih 1 and of all sites in 1979 and 1980 are based on samplings in September.

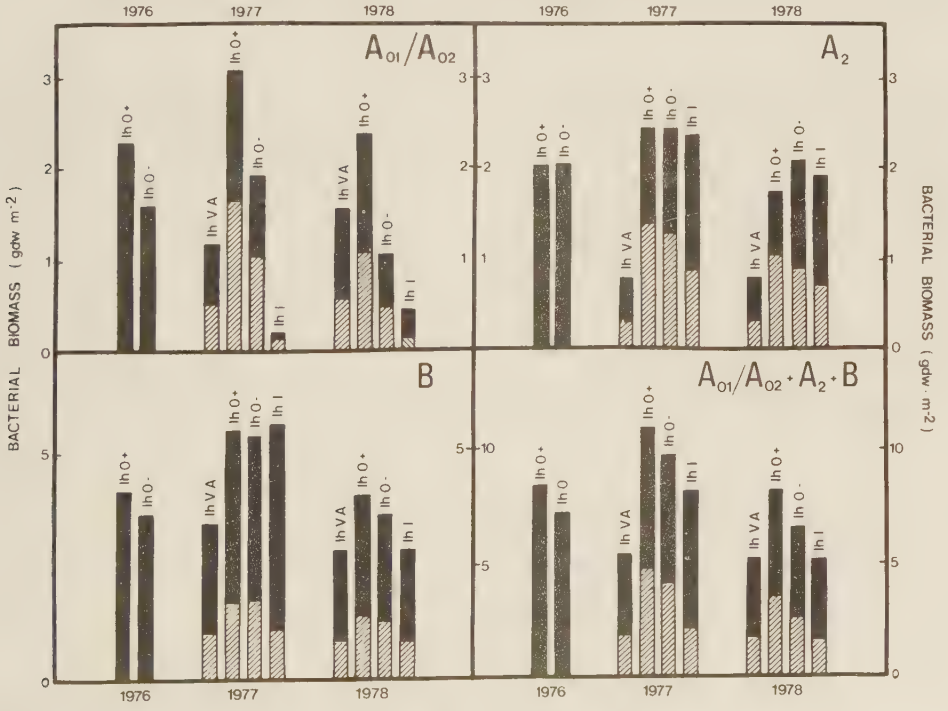

Fig. 1. Annual mean values of AO-stained (entire bars) and FDA-stained (diagonal lines) bacterial biomass in the different soil horizons. Ih VA = mature forest, Ih 0+ = clear-cut area with slash left on the ground, Ih 0− = clear-cut area with slash removed, and Ih 1 = about 10-yr old clear-cut area. In the A_{01}/A_{02} horizon the standard error was 12 and 14% for AO and FDA-stained bacteria, respectively. Corresponding values for the A_2 horizon were 9 and 13%, B horizon 12 and 11%, and the whole profile 8 and 9%, respectively.

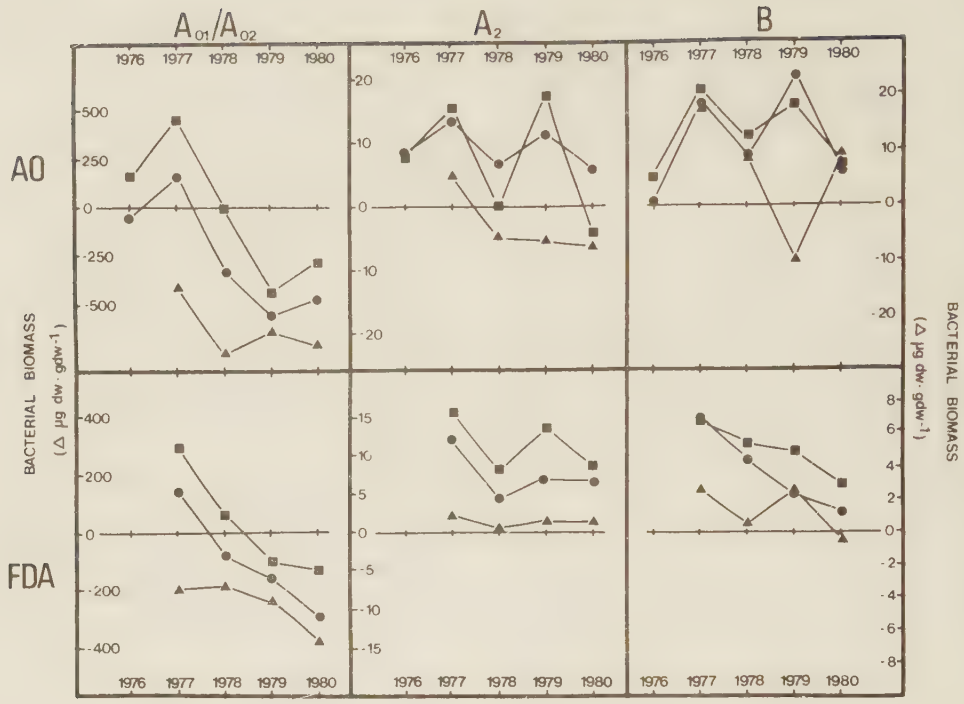

Fig. 2. Biomass of AO-stained and FDA-stained bacteria at sites Ih 0 with slash (■), Ih 0 without slash (●), and Ih 1 (▲) minus the corresponding bacterial biomass at Ih VA ($\Delta\mu$g dw g^{-1} dry soil).

Bacterial biomasses in the A_{01}/A_{02} layer decreased successively at Ih 0 after the second year and approached that at Ih 1 (Fig. 2).

Ih 0 showed somewhat higher values for FDA-stained bacterial biomass in the A_2 horizon than Ih VA (Fig. 2). The difference is present in the AO-stained biomass, too, although not as obvious as for the FDA-stained bacteria. Values for both AO-stained and FDA-stained biomass were similar at Ih 1 and I h VA.

There was a clear difference between the B-horizon of Ih 0 and Ih VA. Bacterial biomass values for Ih 1 varied in an irregular manner. FDA-stained biomass at Ih 0 appeared to decrease compared to Ih VA.

Effects of slash on the ground

At Ih 0 the bacterial biomass in the A_{01}/A_{02} layer was greater in plots with slash left on the ground than in plots without slash (Fig. 1). This difference appeared to increase during the first 3 yr. By the third year there was about twice as much bacteria in plots with slash as in those without slash. This difference in bacterial biomass was found in all 5 yr investigated (Fig. 2).

No statistically significant difference on a m^2 basis was detected between the two treatments in the mineral soil layers at Ih 0 (Fig. 1). There was a tendency towards greater FDA-stained biomass in plots with slash in the A_2 horizon, however. A trend in the same direction was found in the B horizon from 1978 to 1980.

In the entire soil profile plots with slash on the ground appeared to contain greater biomass of bacteria all 3 yr than did plots without slash (Fig. 1). The differences were not statistically significant, however, except for FDA-stained bacteria in 1978 ($P < 0.05$).

Influence of different environmental factors

Partial correlation coefficients were calculated between AO-stained or FDA-stained bacterial numbers and soil moisture content, and loss on ignition. Based on pooled samples on each occasion, significant partial correlation was found between AO-stained bacteria and moisture content (0.69, $P < 0.001$). The FDA-stained bacteria were negatively correlated to loss on ignition (-0.49, $P < 0.05$).

When based on individual soil cores, significant partial correlations were found between AO-stained bacteria and moisture content, with coefficients of 0.36 ($P < 0.01$) and 0.56 ($P < 0.001$) for plots with and without slash respectively. In plots without slash the AO-stained bacteria were correlated to loss on ignition (0.26, $P < 0.05$).

DISCUSSION

The FDA-staining method for counting soil bacteria was used for the first time here in long-term measurements. It was used as an estimation of metabolically-active bacterial biomass. The method was discussed and compared with other microscopic counting methods, including the AO-method (Lundgren, 1981). Here the method gave slightly larger cell sizes than the AO method, on average 0.14 and

0.13 μm^3, respectively. With both methods cell size became smaller with increasing soil depth. Both methods showed the same trends in biomass fluctuations, and thus FDA staining did not give any further information about the bacterial populations. However, the FDA-preparations were more easily examined than the AO-preparations, mainly due to the lack of fluorescence of the soil debris.

It is not possible to regard biomasses, obtained with different direct counting methods, as absolute values, since several methodological sources of error exist. Different preparation and examination techniques give different results, e.g. magnification was shown to influence measurements of hyphal lengths (Bååth and Söderström, 1980). Direct comparisons between different methods (e.g. the absolute values of the FDA and AO methods) should be made very carefully, and also investigations by different laboratories using the same method should be compared with caution. This is illustrated by a comparison of mean bacterial biomass in the A_{01}/A_{02} horizon at I h VA obtained in this work with the AO method, 0.69 mg dw gdw^{-1} of soil, and that of Clarholm and Rosswall (1980), 3.4 mg dw gdw^{-1}.

Clear-cutting strongly influenced bacterial populations in the A_{01}/A_{02} horizon. Since effects of clear-cutting on the bacteria in mineral soil layers were not as obvious, the following discussion is thus mainly limited to the humus (A_{01}/A_{02}) layer.

During the first 2 yr after clear-cutting there was a pronounced increase in bacterial biomass at the clear-cut area (Ih 0) compared to the mature pine stand (Ih VA). During and after the third year bacterial biomass decreased at Ih 0. Plots with slash consistently contained greater amounts of bacteria than those without. The initial effect is in accordance with Laudelout *et al.* (1978), who reported increased viable counts the first year after clear-cutting of a spruce stand. The same result was reported by Grunda (1964) from several clear-cut sites. However, Sundman *et al.* (1978) reported continued increase in bacterial viable counts as long as 7 yr after clear-cutting of spruce forests. It should be noted that their investigation was made with different sites representing the different ages of clear-cutting and not as a time sequence in one area.

The magnitude of increase in bacterial biomass during the first year as an effect of clear-cutting is difficult to assess, since the reference stand was sampled just once this year. Unfortunately, no bacterial analyses were made at Ih 0 before clear-cutting.

Gadgil and Gadgil (1978) found that removal of mycorrhizal roots increased litter decomposition. They suggested that mycorrhiza are suppressive to saprophytic microflora. After clear-cutting the myccorhiza would probably disappear, allowing the saprophytic microflora to proliferate. This may explain the increase in bacterial biomass during the first 2 yr of study at Ih 0.

Chances in nutrient sources might also have been of importance for the changes in bacterial biomass. Root exudation would be expected to decrease after clear-cutting, and would thus not contribute to the increase in bacterial biomass during the first year. The decomposition of the fine root biomass, which was turned over in 1 yr in a young pine stand (Persson, 1980), and coarser root fractions may have been important in providing energy and carbon for the microorganisms during the first 2 yr. Since vegetation in the field layer at Ih 0 disappeared almost completely (Axelsson and Bråkenhielm, 1980), root production would have ceased. This would result in exhaustion of the nutrients available for microorganisms from root litter, which may explain the decrease in bacteria after the second year. The long-lasting increase in bacterial amounts in the investigation by Sundman *et al.* (1978) may in contrast be an effect of heavy growth of grasses at the clear-cut sites. 67% of the ground was covered with *Deschampsia flexuosa* 5–6 yr after cutting, compared to 1% of the intact spruce stand (Huhta, 1976). Grasses are known to have a large root production (Bowen, 1979), resulting in a considerable input of energy into the soil.

The removal and addition, respectively, of slash in the plots at Ih 0 was probably of minor importance for the soil nutrient status. Nykvist (1959) has shown that leaching from pine needles is very slow, and the incorporation of needle litter into the A_{01}/A_{02}-horizon takes several years in this kind of forest.

Soil physical factors like moisture content have been shown to influence bacterial biomass (Clarholm and Rosswall, 1980). Plots at Ih 0 with slash showed reduced evaporation due to shading. This resulted in a higher soil moisture content (on average 280% H_2O of dry soil in plots with slash and 200% in plots without). The partial correlation coefficients indicated a dependence on moisture content for the bacterial populations, which may explain the difference in bacterial biomass between the two treatments at Ih 0. Differences between Ih 0 and Ih VA cannot be explained in this way, however, since the reference stand contained on average 270% H_2O of dry soil.

The differences between the sites in mean temperatures in the A_{01}/A_{02}-layer were less than 1°C (P.-E. Jansson, unpublished results). Also, the differences in intervals of maximum–minimum temperatures were small, about 1°C. Thus, temperature does not appear to have had any important influence on the sizes of bacterial populations.

It is difficult to assess the significance to silvicultural practices of the effects of clear-cutting on soil bacteria. However, decreased bacterial biomass together with decreased fungal biomass (Bååth, 1980) indicate an inability of microorganisms to bind plant nutrients and to reduce possible nutrient loss from the ecosystem during the period of bare land.

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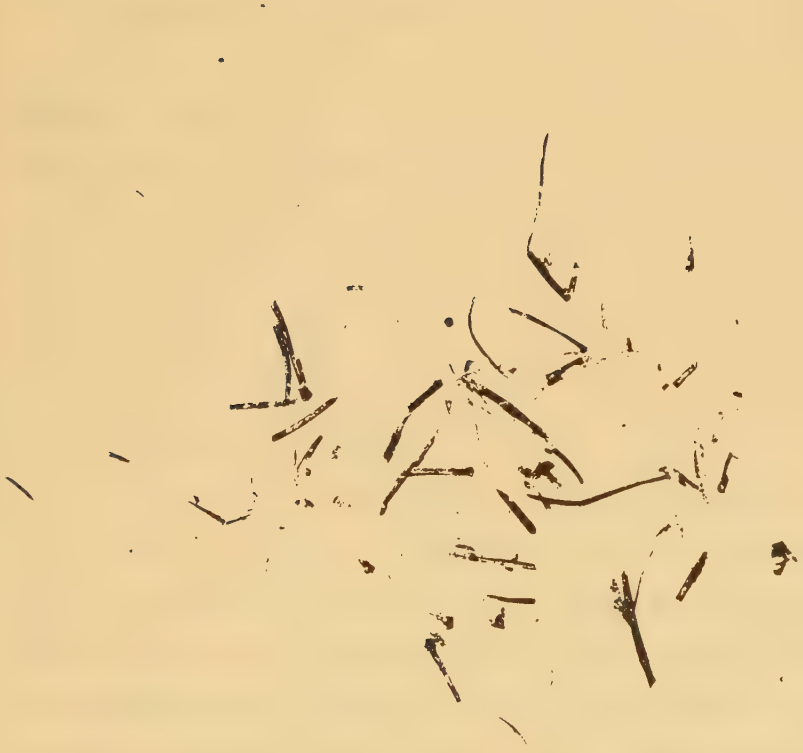

BACTERIAL NUMBERS IN A PINE FOREST SOIL IN RELATION TO ENVIRONMENTAL FACTORS

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Summary - Numbers of soil bacteria stained with acridine orange (AO) or fluorescein diacetate (FDA) were studied for 3 and 2 years respectively. Three pine forest sites were used and both organic and mineral soil layers were included. Different patterns of fluctuation in bacterial numbers were found each year. Significant correlations were demonstrated between AO-stained bacterial numbers and soil moisture content and between FDA-stained bacteria and the accumulated precipitation during a week before the sampling date. In multiple regression analyses 60-80 % of the variation in numbers of AO-stained bacteria could be accounted for by soil moisture and numbers of bacterial-feeding nematodes at the sampling dates, and 30-45 % of the variation in numbers of FDA-stained bacteria was accounted for by precipitation.

INTRODUCTION

Numbers of forest soil bacteria and their dynamics have been studied mainly by plate count techniques. Fehér (1933) studied several european forest soils. Kilbertus et al. (1979) presented data from a forest soil in France, Gray et al. (1974) studied a deciduous woodland in England, Laudelout et al. (1978) two spruce forest sites in Belgium, and Kauri (1982) examined the bacteria in a Swedish beech forest soil. In addition to the numbers of bacteria, Kilbertus et al. (1979) made identifications on the bacterial flora, and Kauri (1983) and Hissett and Gray (1974) gave some data on the physiology of the bacterial populations. Direct microscopical counting of bacteria, using Rossy-Cholodny slides was made by Hoff (1950) and total counts were obtained by Clarholm and Rosswall (1980) using acridine orange staining. They worked in a podzolic soil in a Scots pine stand. Lundgren (1982) also used direct counting methods for the study of clear-cutting effects on the soil bacteria in a pine forest soil.

We have studied numbers of bacteria in communities in pine forest soils for three years, and have attempted to explain the changes in bacterial numbers in terms of environmental factors. In addition to total counts, obtained by the acridine orange staining method, numbers of metabolically-active bacteria were estimated by fluorescein diacetate staining (Lundgren 1981).

MATERIALS AND METHODS

The site

The site was a Scots pine (Pinus sylvestris L.) forest at Ivantjärnsheden in Central Sweden. The soil is an iron podzol on glacifluvial sandy sediment. Annual mean temperature is 3.8°C, and annual precipitation is 607 mm at Åmåtsbruk, 15 km N of the site (Swedish Meterological and Hydrological Institute). The site was described in detail by Axelsson and Bråkenhielm (1980).

A mature pine stand (IhVA), about 120 years old, and two treatments of a clear-cut area (Ih0), cut in March-April 1976, were used. At the Ih0 site the two treatments were one with slash (twigs and branches, including needles) removed (Ih0-), and one with double the normal amount of slash left on the ground (36 ± 4.5 tonnes dw ha^{-1}, Ih0+).

All precipitation and temperature data used were provided by P.-E. Jansson, Swedish Coniferous Forest Project, Uppsala, Sweden. The temperature measurements were made at 3 - 5 cm depths. The methods for the climatic registrations were described by Perttu et al. (1980). The temperature data of Ih0 in 1978 were computer simulated data (Halldin et al., 1980).

Sampling

Samples were taken every month during the growing seasons (from May to October-November). In the clear-cut areas (Ih0) samples were taken for three years, 1976 to 1978, and in the mature pine stand (IhVA) for two years, 1977 to 1978. On two occasions during the winter, in February and March 1978, samples were taken at IhVA under a ca 0.6 m thick snow cover.

Each time six cores were taken for each field treatment. The soil layers, humus layer (A_{01}/A_{02}), elluvial (bleached) mineral soil layer (A_2), and illuvial (enrichment) mineral soil layer (B), were analysed separately. Further details of the treatments of the soil cores are given by Lundgren (1982).

Analyses

The bacterial community was analysed using two direct microscopical methods. The total counts, (living and dead bacterial cells) were made with acridine orange (AO) stained smears (Trolldenier, 1972, as modified by Clarholm and Rosswall, 1980). Metabolically-active bacteria were estimated with the fluorescein diacetate (FDA) staining method (Lundgren, 1981).

Bacterial cell volumes were calculated after size classification. Biomasses were calculated by using a cell density of 1.3 g cm^{-3} and a dry weight based on 20% of the wet weight

(Faegri et al., 1977).

Dry weights of soil were obtained gravimetrically (105°C) and organic matter content was estimated as loss on ignition (LOI, 850°C).

Statistical analyses

Multiple linear regressions were calculated with AO-stained and FDA-stained bacterial counts as dependent variables respectively. In these analyses of the A_{01}/A_{02}-layer moisture content, LOI, and nematode numbers (data from Sohlenius, 1982) were used as independent variables. Campbell and Biederbeck (1976) made regression calculations between bacterial numbers and different temperature variables including changes in temperature. Diurnal and weekly mean temperatures, and diurnal and weekly temperature fluctuations ($t_{max}-t_{min}$) were thus also checked for any correlation with bacterial numbers. No relationship between bacteria and any of the temperature variables were found. These variables were not further analysed. In the mineral soil layers only moisture content and loss on ignition were used in the multiple linear regression analyses, since data on nematodes in specifically these soil layers were not available. The three treatments were analyzed separately, and $\log_{10}$-transformed data were used. For the AO-stained bacteria at Ih0, data from separate cores were used in the analyses (1976 - 1977). For AO-stained bacteria at IhVA, and FDA-stained bacteria at all three sites, data from pooled

samples were used. Partial correlation coefficients were calculated on the same data between the three variables finally used in the multiple regressions, bacterial numbers, moisture contents and nematode numbers.

Using accumulated precipitation (in mm) for 7 days before sampling as an independent variable, linear regressions and correlation coefficients were calculated for the numbers of AO- and FDA-stained bacteria respectively. All three soil layers were analyzed. Precipitation was not included in the multiple linear regressions since these analyses then would have been based on too few sampling occasions due to missing data.

RESULTS

The bacterial biomass differed considerably between both the sampling dates and between the soil layers. The range of the biomasses are given in Table 1, where the mean values of the ratio between the acridine orange stained and the fluorescein diacetate stained bacteria are also given. The low biomass in the A_2- and B-layers is mainly due to the low organic matter content, and the lower FDA/AO ratio in the B-layer is due to a decline in the FDA-stained bacteria, while the numbers of AO-stained ones remained constant. This is consistent with the sharp decrease in viable counts (plate counts) with soil depth usually found (e.g. Kauri 1982). Since the biomass values are based on calculations using a mean value of specific bacterial cell weight, only the bacterial numbers will be considered in the following presentation.

The fluctuations over the years in the total counts were similar at the three sites in the organic soil layer (Fig. 1). However, when comparing the different years, the pattern of the fluctuations was different. The first year there was a dramatic drop in AO-stained bacterial numbers from the end of April to the beginning of May. For the rest of that year, the numbers were rather stable. The second year low values were obtained in early summer, peaking in the autumn. The highest values this year were obtained at the end of the sampling period in November. In the third year, rather constant values were obtained during the summer, but a peak was

reached in September. There was only one feature in common for all three years, namely the low bacterial counts during the summer months, in June or July. This coincided with low values of soil moisture content.

The FDA-staining method was not used during the first year. The numbers of FDA-stained bacteria fluctuated in a similar way in the different sites (Fig. 1). This was most obvious comparing Ih0 with and without slash. As with AO-stained bacteria, the fluctuation in the number of FDA-stained bacteria was greater in the second than in the third year. The fluctuations in AO- and FDA-stained bacteria were not well correlated as indicated by the insignificant correlation coefficients from -0.2 to 0.6.

In the mineral soil layers the fluctuations were smaller. The ranges of AO-stained bacterial numbers were 0.7-2.2 x 10^9 g^{-1}dw, and 0.6-2.6 x 10^9 g^{-1}dw in the A_2- and B-layers respectively. For the FDA-stained bacteria the ranges in the same soil layers were 0.2-1.2 x 10^9 g^{-1}dw, and 0.1-0.7 x 10^9 g^{-1}dw. There was covariation between the sites and, as for the organic soil layer, there was a different fluctuation pattern during the 3 years. During the second year the fluctuations were more pronounced than for the third year. This phenomenon was found both for AO-stained and FDA--stained bacterial counts. In the first year, the dynamics were almost identical in the two treatments at Ih0, especially in the A_2-layer. Any feature common to all three years, like low values during summer, was not found in these soil layers.

During the winter months in 1978, higher AO-stained bacterial numbers were found in March compared to February; the values increased from 34.8, 1.1 and 0.8 x 10^9 g^{-1}dw to 43.8, 1.4 and 1.2 x 10^9 g^{-1}dw in A_{01}/A_{02}, A_2- and B-horizons respectively. In the A_{01}/A_{02}-layer these values were high in comparison to those obtained during the growing seasons. In the A_{01}/A_{02}-layer the number of FDA-stained bacterial decreased during the same period (from 7.1 to 2.3 x $10^9 g^{-1}$ dw), while no changes were found in the other horizons.

In the multiple linear regression analysis loss on ignition was insignificant in all regressions, and was hence omitted. The regressions were then recalculated with moisture content and nematode numbers as independent variables in all equations to allow comparison between different treatments. As dependent variables bacteria stained with the two different methods were used.

All the resulting equations explained a significant part of the variations in both AO- and FDA-stained bacterial numbers in the A_{01}/A_{02}-layer (Table 2). The R^2-values varied between 0.58 and 0.82. However, only those equations based on data from separate soil cores, i.e. those for AO-stained bacteria at Ih0, showed significant partial regression coefficients.

Significant positive regression coefficients, ranging between 0.127 and 0.160 ($\log_{10}$-values) were found between the 7-days precipitation and numbers of FDA-stained bacteria in the A_{01}/A_{02}-layer at Ih0 without slash and IhVA (Table 3). The same tendency was found in the linear regres-

sion on data from Ih0 with slash although it was not significant. No significant regressions were found between 7- days precipitation and AO-stained bacterial numbers, since the regression coefficients were considerably lower (0.041-0.081).

The partial correlation coefficients and the correlation coefficients for the A_{01}/A_{02}-layer are also given in Table 2 and 3, respectively. The levels of significance of these coefficients were essentially the same as for the regression coefficients. The values of the partial correlation coefficients between FDA-stained bacteria and nematodes were high although not significant.

No multiple linear, linear regressions or correlations for the mineral soil layers showed any significance.

DISCUSSION

Many factors influence growth and survival of soil bacteria. The most obvious one is availability of nutrients, but other factors like soil water regime and temperature at a given time are certainly also of importance, as well as changes in these environmental variables. Major changes in such variables can generally be shown to follow some annual pattern, but obviously, differences between different years might be considerable (see Fig.1 and Axelsson and Bråkenhielm 1980). Consequently, one might expect some seasonal fluctuation in soil bacterial growth and biomass, but also somewhat different patterns in fluctuation between different years.

Such seasonal fluctuations have been found by Clarholm and Rosswall (1980) in the same pine forest as we have investigated (IhVA). They found a small peak in spring and a large one in autumn. Two studies in Belgium an France (Laudelout et al. 1978, Kilbertus et al. 1979) also demonstrated changes in the soil bacterial numbers over the year in coniferous forest soils. Fehér (1933) found seasonal variation in bacterial numbers in several european forest soils, and Kauri (1982) found peaks in spring and autumn in a Swedish beech forest soil. However, the two latter investigations were the only ones which lasted for more than one year. In contrast to these studies Gray et al. (1974) did not find any regular seasonal fluctuations in bacterial

counts in their 2-year-study. This is in accordance with our results with quite different patterns of fluctuations over the 3 different years. Only Clarholm and Rosswall (1980) made microscopical estimations of the numbers of bacteria. All other mentioned investigations were based on plate counting techniques. This makes detailed comparisons less fruitful. However, the results obtained in all these studies clearly emphasize the importance of extending this kind of investigation for more than 1 year. In addition, since the short-term changes in environmental factors often are large (see below), the seasonal changes might be obscured when samples are only taken every month. If the numbers of soil bacteria were estimated more often it is possible that seasonal fluctuations could be detected.

It appears from the monthly estimations in our study that short-term changes in environmental conditions might be of major importance for the changes in soil bacterial numbers. This assumption is based on (i) the irregularity of the fluctuations (Fig.1), also found by Gray et al. (1974), and (ii) that in spite of these irregularities there was a very good covariation between the numbers of bacteria at the three sites studied. Precipitation causes rapid changes in the soil environment. In addition to wetting of the soil, rainfall results in increased availability of nutrients in soil (Clarholm and Rosswall, 1980). Due to evaporation, the soil water content may rapidly decrease again, so that no detectable changes in soil moisture can be found just a few days later. Soil moisture is, in addition to precipitation,

dependent on evaporation rate, which in turn is dependent on e.g. air temperature and humidity, these latter factors being seasonally dependent. Precipitation may thus be a better indicator of short-term changes in the soil environment than soil moisture content at a specific date, since the latter might be more seasonally dependent. It is therefore quite reasonable that correlation of FDA-stained bacterial numbers and precipitation was found, since the FDA-staining method represents an attempt to quantify metabolically--active bacteria selectively (Lundgren 1981). Campbell and Biederbeck (1976) found an increase in viable counts shortly after rainfall, and our results are thus in accordance with theirs. On the other hand, AO-stained bacteria, which include active, inactive and dead cells, were not significantly correlated to precipitation, but rather to soil moisture content. This is consistent with the results of Clarholm and Rosswall (1980) who found no clear relationship between numbers of AO-stained bacteria and precipitation, although they found a peak in bacterial biomass shortly after rainfall.

No correlation was found between moisture content or precipitation and bacterial counts in the mineral soil layers. The mineral soil is more constant as far as moisture and temperature are concerned. This is due to the shelter provided by the humus layer. In addition, all changes are slow compared to the soil layers above. This in turn affects the bacteria, in that the fluctuations in numbers are slower and of less amplitude, especially in deeper soil layers (B).

This phenomenon was also found by Clarholm and Rosswall (1980). Söderström (1979) demonstrated the same effect for FDA-active fungal biomass. This makes any correlation between bacterial numbers and environmental factors difficult to detect.

During the winter we noticed an increase in AO-stained bacteria, but a decrease in numbers of FDA-stained cells in the organic soil layer. The decreasing numbers of FDA-active cells indicated a low biological activity which is reasonable since the soil temperature was a few degrees below zero. At this temperature, inactive or dead cells seemed to accumulate, indicated by the increase in numbers of AO--stained cells. In 1976 the numbers of AO-stained bacteria at Ih0 decreased rapidly from the end of April to the beginning of May. In April most of the soil samples were frozen, particularily those from the plots with slash left on the ground. In May the soil had thawed. The rapid decrease in numbers during this period might well be explained by a rapid decomposition of accumulated dead cells in the thawed soil. This explanation is supported by the results of Nelson and Visser (1978) who by viable counts found a peak in numbers of bacteria during the thaw. However, Biederbeck and Campbell (1971) found thawing lethal to the microflora in laboratory experiments with soil. A quite different explanation to the rapid decrease in bacterial numbers could thus be that the cells were killed during thawing and then rapidly disintegrated. To clarify the effects of thawing in the field further investigations are needed.

Soil temperature or changes in temperature did not seem to influence the bacterial numbers to any detectable degree. This is in accordance with the results of Waksman (1927) and Campbell and Biederbeck (1976), who found that temperature is not very important compared to soil moisture in influencing changes in microbial populations. Nevertheless, temperature is obviously of major importance in governing the total activity of the microorganisms, as pointed out e.g. by Bunnell et al. (1977). Assuming a constant respiration rate per bacterial cell at a given temperature and a Q_{10}-value of 3, bacterial respiration was calculated for each sampling occasion. In contrast to bacterial numbers, the highest respiration rate was obtained in summer for each of the 3 years studied. This was the case irrespective of the biomass estimation, FDA-or AO-staining procedures, was used as a basis for the calculations. Identical effects of temperature on FDA-active fungal biomass was noticed by Bååth and Söderström (1982).

Not only abiotic factors may affect the bacterial activity. The soil fauna is also known to influence the bacterial populations, both by grazing (Anderson et al., 1978; Bååth et al., 1978, 1981), and by other mechanisms, e.g. increasing the availability of organic matter by comminution (Swift et al., 1979). The main grazers on soil bacteria are protozoa and nematodes (Anderson et al., 1978). Simultaneously with the bacterial analyses, the nematode populations were investigated (Sohlenius, 1982). At IhVA 54% of the nematode community was comprised of bacterial feeders

(Sohlenius, 1979) and about the same proportion was also found at Ih0 (Sohlenius, pers. comm.). Although not always significant, positive partial correlations were found between bacterial numbers and nematode abundance. This implies that high bacterial numbers support high nematode numbers. However, the causal reltionships between these two organism groups are more complicated. Bååth et al. (1978) and Bååth et al. (1981) found that nematode numbers were positively correlated to bacterial activity using respiration rate as a criterion, but not to bacterial numbers. In short term studies in a pine forest soil (IhVA) and a laboratory study, Clarholm (1981) found a rapid decrease in AO-stained bacterial biomass, while protozoan biomass increased. The protozoa are a considerable part of the bacterial feeding fauna in this soil (Clarholm 1981). It is thus difficult from the field data of our investigation to assess the mechanism of the ifluence of nematodes on the bacterial numbers, and to what degree the bacteria are affected by the nematodes. To obtain a better picture of the effects of the bacterial feeding fauna, estimations of the protozoan biomass should have been included in the investigation.

We conclude that from our study moisture seems to be the main factor in determining the dynamics of the soil bacterial populations in natural podzolic soils. The total counts (AO-stained bacteria) were related to moisture content, while the FDA-active fraction of the bacterial community was better correlated to rainfall. In addition, bacterial feeders probably have a substantial effect on the bacterial populations in the field.

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TABLE 1. Range of the acridine orange (AO)-stained and the fluorescein diacetate (FDA)-stained biomasses and the ratio between them in three soil layers. Mean values from two clear-cut areas and a mature forest are shown.

Soil layer	Biomass range (ug dw g^{-1} dw)		FDA/AO ($\bar{X}$)
	AO	FDA	
A_{01}/A_{02}	300-1500	130-750	0.52
A_2	23-74	7-42	0.54
B	18-80	6-25	0.34

TABLE 2. Equations from multiple linear regression analyses for numbers of AO-stained or FDA-stained bacteria in the organic soil layer (A_{01}/A_{02}) at three sites (Ih0- and Ih0+ = clear cut areas without and with slash respectively, IhVA = mature forest). Based on $\log_{10}$-transformed data. r = partial correlation coefficient. [a]= based on separate soil cores, the rest on pooled samples.

				$\%H_2O(X_1)$		nematode numbers (X_2)
AO-stained bacteria						
Ih0-[a]	Y	=	8.954	+ 0.387 X_1	+	0.158 X_2
n = 66	r =			0.55***		0.35**
R^2 = 0.67, $F_{(R^2)}$ = 63.1**						
Ih0+[a]	Y	=	8.979	+ 0.287 X_1	+	0.255 X_2
n = 60	r =			0.33**		0.48***
R^2 = 0.58, $F_{(R^2)}$ = 38.9**						
IhVA	Y	=	9.514	+ 0.213 X_1	+	0.108 X_2
n = 8	r =			0.58 n.s.		0.49 n.s.
R^2 = 0.82, $F_{(R^2)}$ = 11.0*						
FDA-stained bacteria						
Ih0-	Y	=	9.151	- 0.318 X_1	+	0.605 X_2
n = 10	r =			-0.34 n.s.		0.62 n.s.
R^2 = 0.66, $F_{(R^2)}$ = 6.73*						
Ih0+	Y	=	8.846	- 0.021 X_1	+	0.462 X_2
n = 10	r =			-0.02 n.s.		0.66 n.s.
R^2 = 0.66, $F_{(R^2)}$ = 6.86*						
IhVA	Y	=	9.164	+ 0.089 X_1	+	0.221 X_2
n = 7	r =			0.15 n.s.		0.48 n.s.
R^2 = 0.82, $F_{(R^2)}$ = 8.81*						

TABLE 3. Linear regressions between bacterial numbers (Y) and precipitation during seven days before sampling (X), based on $\log_{10}$-transformed data. For explanation of symbols see Table 2. r = correlation coefficient.

AO-stained bacteria			
Ih0-	Y	= 10.144 +	0.081 X
	F = 4.21 n.s.	r = 0.46*	n = 18
Ih0+	Y	= 10.334 +	0.041 X
	F = 0.75 n.s.	r = 0.21 n.s.	n = 18
IhVA	Y	= 10.214 +	0.048 X
	F = 0.67 n.s.	r = 0.28 n.s.	n = 10
FDA-stained bacteria			
Ih0-	Y	= 9.837 +	0.160 X
	F = 5.61*	r = 0.62*	n = 11
Ih0+	Y	= 10.010 +	0.133 X
	F = 4.18 n.s.	r = 0.56*	n = 11
IhVA	Y	= 9.775 +	0.127 X
	F = 6.93*	r = 0.68*	n = 10

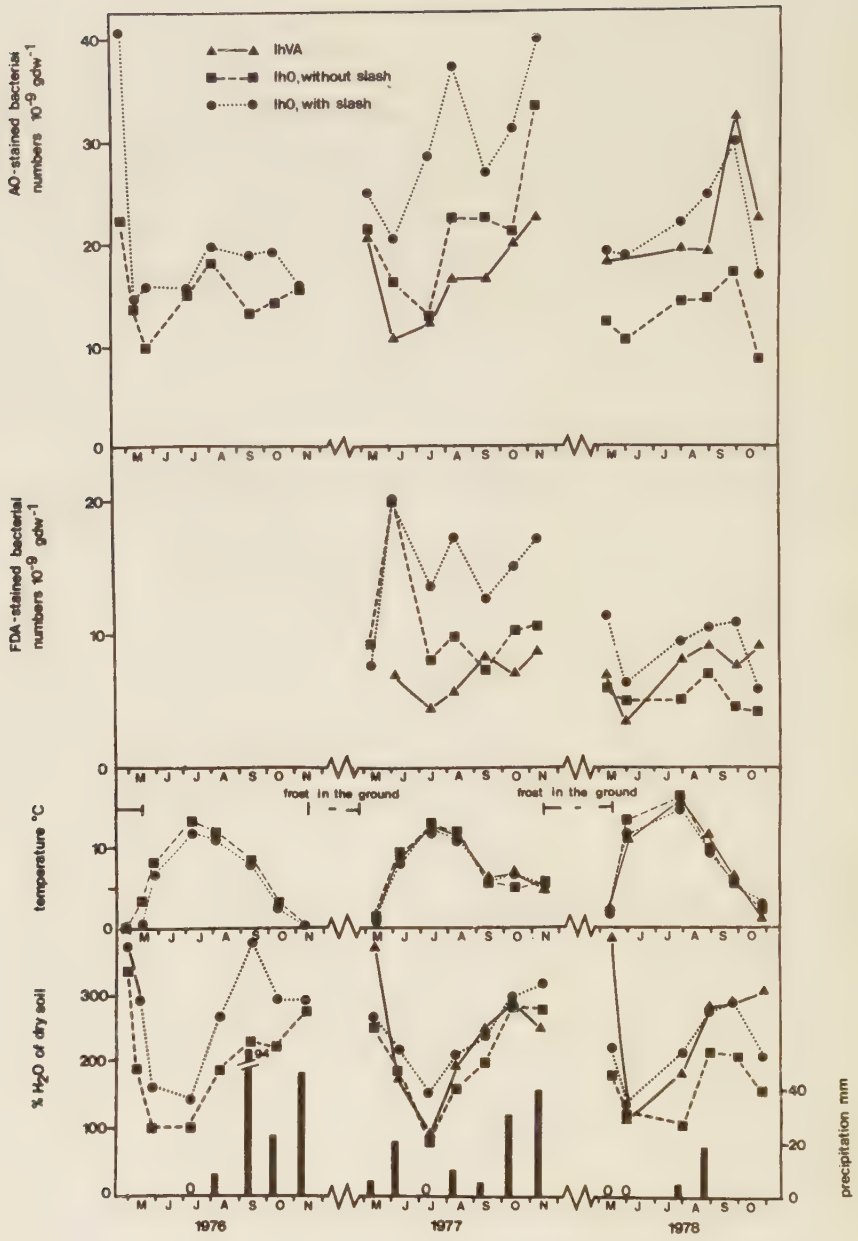

Figure 1. The numbers of AO- and FDA-stained bacteria, temperature, and moisture content are shown for the A_{01}/A_{02}-layer at three different sites; mature pine stand (IhVA), and two treatments in the clear-cut area (Ih0). The average standard error was 14 % for AO-stained bacteria and 15 % for FDA-stained bacteria. Accumulated precipitation during the 7 days before sampling is also shown. No precipitation during this period is indicated with zero.

V.

IMPACT ON SOIL BACTERIAL NUMBERS AND ACTIVITY BY A CEPHALOBID NEMATODE

by

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ABSTRACT

Sterilized podzolic soil was inoculated with a mixed soil bacterial population in two series, one with and one without bacterial feeding nematodes (Acrobeloides nanus). The sizes of bacterial and nematode populations and respiration rate were estimated over eight weeks. Bacteria were counted with two direct microscopical methods (acridine orange, AO,- and fluorescein diacetate, FDA,-staining). Bacterial growth and activity were greatly stimulated by the nematode population, as it was rapidly expanding. This was indicated by the higher FDA-stained bacterial numbers and CO_2-evolution in the series with nematodes in comparison to that without. Later, when the dense nematode population (up to 19000 animals g^{-1} dry soil) stagnated, the respiration rate was still greater in nematode-treated soil, but the numbers of both FDA- and AO-stained bacteria were lower in the treatment with nematodes compared to that without animals. The presence of nematodes reduced the dominance of a coccoid bacterial strain over three strains of rods. Possibly, this was caused by reduced intraspecific competition between the bacteria due to nematode predation.

INTRODUCTION

Bacterial feeding nematodes can exert a strong influence upon bacterial populations and soil respiration. This has been shown in a number of microcosm experiments conducted with soil from a shortgrass prairie (Anderson et al. 1978, Coleman et al. 1978), from a coniferous forest (Rosswall et al. 1977, Bååth et al. 1978, 1981a), and with sewage sludge (Abrams and Mitchell 1980) as substrate. Bååth et al. (1981a), and Anderson et al. (1978, 1981) reported a reduction in bacterial numbers and increased rate of nitrogen mineralization in the presence of nematodes. Positive correlation between nematode abundance and soil respiration rate were observed (Bååth et al. 1978, 1981a, Clarholm et al. 1981). The increased respiration rate could not be explained by nematode respiration only. A greater part of it probably originated from microorganisms, mainly the bacteria. Since no correlation between acridine orange (AO)-stained bacterial numbers and respiration rate could be observed, the metabolic activity of the bacteria was not reflected in their biomass (Bååth et al. 1978, 1981a). In a field investigation, however, positive correlation between fluorescein diacetate (FDA)-stained bacterial numbers and nematode abundance was found (Lundgren and Söderström, in press). This implies that the metabolically active part of the bacterial populations (measured as FDA-stained bacteria) may be related to the respiration rate in laboratory experiments.

The aim of the present work was to study the dynamics of a nematode population (Acrobeloides nanus) and FDA- and

AO-stained populations of bacteria over time in relation to changes in respiration rate in a laboratory investigation.

MATERIALS AND METHODS

Soil

A podzolic Scots pine (Pinus sylvestris L) forest soil on sandy sediment from Torrmyra, Southern Sweden, was used. The organic soil layer (A_{01}/A_{02}, pH 3.8 in H_2O, loss on ignition 82.5 %) and enrichment (illuvial) soil layer (B, pH 4.4, loss on ignition 3.3 %) was mixed in a w/w ratio of 1:2. The soil mixture was thoroughly blended with 1.5 % CaO to increase the pH-value, which would allow a rapid proliferation of the bacteria.

Organisms

The bacterial feeding nematode Acrobeloides nanus (de Man 1880) Anderson, 1968 was isolated from an iron podzol under Scots pine forest at Jädraås, Central Sweden. The nematodes were cultured on agar plates with Nigon´s medium (Nigon 1949). The cultures contained four different strains of bacteria, one coccus and three different rods. These bacteria were used in the experiments.

Experimental procedures

The limed soil mixture was divided into 300 ml bottles, 10 g dw in each, and autoclaved 3 x 45 min at 110°C for 24 h. The pH-value after sterilization was 5.9. The moisture content was adjusted to 80 % of water holding capacity (WHC) with sterilized distilled water.

Half of the bottles were inoculated with bacteria and nematodes and the rest with bacteria only. Organisms from two agar plates, 19- and 31-days old, were suspended in ca 10 ml sterilized tap water and mixed. One series of bottles was inoculated with this suspension, giving 100 nematodes + 30 eggs and 1 x 10^8 bacteria per bottle. For the series without nematodes, the suspension was filtered through a membrane filter (Millipore 5 µm pore size) before inoculation, excluding the nematodes and eggs from the filtrate. To additional bottles corresponding amounts (0.1 ml) of sterilized tap water were added. These bottles were used for sterility controls and measurements of nonbiological CO_2-evolution (see below). The bottles were incubated horizontally, which facilitated gas exchange. Incubation temperature was 20°C.

Analyses

Two experiments were performed. In the first experiment 3 bottles from each treatment and 1 control bottle were sampled at each of 10 sampling occasions. The second experiment differed in that 18 bottles were examined after 22 and after 65 days in the nematode-treated bottles. In the treatment free of animals 3 and 4 bottles were analysed at the two sampling occasions.

For respiration measurements, the bottles were closed, concentration CO_2-measured, and the bottles incubated for 20 h. The CO_2-concentration was again measured and the respiration rate calculated. CO_2-evolution of the sterile bottle was substracted to obtain the biological CO_2-

-evolution. CO_2-concentration was measured by gas chromatography (Bååth et al. 1981b). The bottles were weighed for calculation of moisture loss through evaporation. This was possible since weight loss due to respiration was negligible. The pH-values were measured in water suspensions (1:2 w/v soil:water ratio).

Numbers of fluorescein diacetate (FDA)-stained bacteria (as a measure of metabolically active bacteria), and of acridine orange (AO)-stained bacteria (total amount of bacteria) were estimated with fluorescent microscopical methods (Lundgren 1981). Biovolumes were obtained after size classification of the bacterial cells.

The nematodes were extracted with a wet funnel method (Sohlenius 1979). The animals were then microscopically counted, length classified, and the frequency of egg--carrying animals determined. Nematode biomass was calculated according to Andrassy (1956).

The results of respiration measurements, FDA- and AO--stained bacterial countings, and measurements of average cell volumes were statistically analysed by two-factor ANOVA, using time of examination and nematode treatment as the factors. The respiration values were log-transformed prior to statistical analysis. Comparisons between mean values in the two treatments were made with Tukey´s honestly significant difference method in the first experiment, and the Tukey-Kramer method in the second one because of unequal sample sizes (Sokal and Rohlf 1981). All comparisons were made at the 95 % significance level. Linear regressions were calculated on the data of mean cell volumes.

RESULTS

During the period of incubation the moisture content decreased from 80 % to 50 and 62 % of WHC in the treatments with and without nematodes respectively in the first experiment. In the second experiment the moisture content decreased to 36 and 32 % of WHC, respectively. The pH--values increased to about 6.4 in both series, but remained rather constant (5.8) in the sterile soil.

Results presented in Figs 1-4 refer to the first experiment. The abundance of nematodes was low in the beginning of the experiment (4 to 15 specimens g^{-1} dry soil during the first eight days). Not until the 17th day did any pronounced increase occur (Fig. 1). At this time about 600 nematodes g^{-1} dw were found. Then the abundance rapidly increased till the 31st day. The rate of increase then slowed, but after 52 days a slight increase in nematode numbers was still found. At this time about 19000 animals g^{-1} dw were found.

There was a flush in CO_2-evolution during the first days in both treatments (Fig. 2). After the second day the respiration rate decreased progressively throughout the experiment. After 17 days the series with nematodes showed greater respiration rate compared to the series without animals. However, this difference was significant only at day 31 and 52.

The FDA-stained bacterial numbers increased in both treatments during the first weeks, slightly faster in the treatment without nematodes (Fig. 3A). Peak values

obtained at 17 days in the series without, and at 24 days in the treatment with nematodes. At day 24 there were significantly more bacteria in the nematode-treated soil than in the soil without animals. The numbers of FDA--stained bacteria decreased towards the end of the experiment. This was most pronounced in the series with nematodes, which had significantly lower FDA-stained bacterial numbers at day 38 and 52 compared to the series without nematodes.

After a peak value at 17 days the AO-stained bacterial numbers decreased; lower values were found in the nematode treatment throughout the experiment (Fig. 3B). The decrease was simultaneous with the first observation of any significant nematode abundance (Fig. 1).

The mean cell volumes of FDA-stained bacteria decreased in the series without nematodes during the experiment, resulting in significantly smaller cell sizes at the three last sampling times (Fig. 4). In the treatment with nematodes fairly constant mean values were obtained throughout the experiment; linear regression analysis resulted in a line with an insignificant slope ($Y = 0.623 - 0.0008 \times X$, μm^3 as the unit) in the nematode treatment. However, the treatment free of animals had a significant slope ($Y = 0.765 - 0.0086 \times X$, $p < 0.001$). The decreasing mean sizes of bacteria were mainly due to an increasing dominance of cocci in the treatment without nematodes. In the FDA-stained preparations three groups of bacteria were distinguished; one group of cocci with a diameter of about 0.8 μm, and two groups of rods with

mean sizes of 0.7 x 1.5, and 0.8 x 3 µm. The last group probably consisted of two strains of rods. When first examined after 8 days, the bacteria comprised 60, 10 and 30 % of respective groups. At the end of the experiment the distribution was changed to 75, ca 0, and 25 %, respectively, in the nematode treatment, but to 92, ca 0, and 8 % in the treatment without animals.

To evaluate the state of nutrition and activity of the nematode population, changes in length class composition and adult status were examined. The frequency of adults dropped from 10-25 % during the first four examinations to 3-5 % during the last three samplings. During the first five days about 63 % of the adults were egg-carrying, and at 17 days 50 % still contained uterine eggs. Later, this proportion dropped to 20 % at 24 days, and thereafter no egg-carrying adults were found. The composition of length classes also changed (Fig. 1). During the period of low and moderate nematode densities, there was a great proportion of long animals. On the three last samplings the composition was rather static, with a dominance of short animals and an absence of very long ones.

In the second experiment (Table 1) the results were essentially the same as in the first experiment. The bacterial numbers, both FDA-stained and AO-stained ones, were greater overall in the second experiment, while the respiration rate was about the same as in the first one. At 65 days, the respiration rate in the nematode treatment, and the numbers of FDA-stained bacteria in the treatment without nematodes were signifi-

cantly greater. The AO-stained bacterial numbers had a reversed relationship compared to the first experiment at 22 days, i.e. greater numbers in the series with nematodes. However, the difference was not significant. At the end of this experiment the numbers of nematodes were about half of that at the end of the first experiment.

DISCUSSION

All microcosm experiments so far published indicate great influences by bacterial feeding nematodes on bacterial numbers. However, different responses have been found. A pronounced reduction in abundance of bacteria has been observed (Rosswall et al. 1977, Bååth et al. 1981a, Coleman et al. 1977), and large reductions have also been described in water suspensions (Wilt et al. 1973, Nicholas and Viswanathan 1975). In the present experiments the bacterial numbers initially increased in the presence of nematodes, but after further incubation the numbers were reduced below the numbers in the nematode free series. This agrees with the results obtained by Anderson et al. (1978), who noted that bacterial densities were slightly higher after 17 days of nematode feeding, but were significantly reduced after 24 days compared to the nematode free treatment. Quite different results were obtained by Abrams and Mitchell (1980) working with sewage sludge. They found greater bacterial populations in the presence of nematodes throughout the whole experimental period (56 days at 22^{o}C) in most of their experiments. They explained these greater populations as stimulation of bacterial growth by increased

oxygen availability, due to nematode movements in an otherwise partly anaerobic substrate. In our experiments the substrate was a loose soil mixture with good aeration so that oxygen was not limiting metabolic activity and growth.

The nematode supplied series of the first experiment (Figs 1-4), could preferably be divided into three different phases, based on nematode growth. During the initial phase, covering the first 11 days, the nematode abundance was very low. The tendency of lower bacterial numbers and respiration rate in the nematode treatment during this time was not due to effects of nematode consumption.

The second phase (17 to 31 days) was characterized by a rapidly expanding nematode population. During this period the growth and activity of the bacteria was stimulated by the presence of nematodes. This was indicated both by an increase in FDA-stained bacterial numbers and respiration rate in this series. The bacteria were calculated to account for 85 % of this increase in respiration rate. A possible explanation of the stimulatory effect could be increased dispersion of the bacteria by nematode movements, which made more soil derived nutrients available (Abrams and Mitchell 1980). Another explanation could be excretion of easily available nutrients by the nematodes. Anderson et al. (1983) found substantial amino acid production in connection with expanding nematode populations, which they thought greatly stimulated bacterial activity. In the present case, the carbon budget calcu-

lations also indicate that a considerable amount of organic compounds was defecated by the nematodes (Table 2).

During the last phase (31 to 52 days) the nematode abundance was very high, but the growth rate was low. Although the numbers of both FDA- and AO-stained bacteria were lower in the series with nematodes compared to the one without, the respiration rate was higher in the nematode treatment. This difference was, however, accounted for by the calculated nematode respiration in the last two examinations. In this phase, these results indicate that the predation pressure on the bacteria increased in relation to stimulatory effects, compared to the second phase. This is quite resonable partly because of the high nematode numbers and partly because the system seemed to stagnate, e.g. due to nutrient limitation. This was indicated by the ceased nematode growth, length class distribution of the nematodes (see below), and low respiration rate during this period. However, the respiration rate per bacterial cell was still enhanced by the presence of nematodes. After 52 days the respiration rate per FDA--stained bacterium was about 50 % greater than in the series without animals.

A carbon budget for the system was calculated (Table 2). These cumulative calculations were done separately for the first 11 days, where the influence of nematodes on the carbon flow was negligible, and for a second period covering days 17 to 52. During this second period quite an important amount of carbon fluxed through the nematode population (Table 2). The total CO_2-evolution was 47 %

higher in the presence of nematodes. This value is close to the 50 % enhancement in the presence of nematodes found by Coleman et al. (1978) and Anderson et al. (1981). When subtracting the calculated nematode respiration, the increase in bacterial respiration was about 22 %.

When comparing the rate of nematode consumption with bacterial production a great proportion of C from the latter was used by the nematodes (Table 2) during the period 17 to 52 days. During the last two examinations the consumption even exceeded the calculated bacterial production. This may, however, be an overestimation. During periods of food shortage Acrobeloides might develop quiescent forms (Anderson and Coleman 1981). The absence of egg-carrying adults and the high percentage of animals in the smallest size classes support the view that the nematode population was in a phase of stagnation at the last examinations (cf Sohlenius 1973). Furthermore, the nematode consumption may not have consisted of bacterial cells exclusively. It is possible that the nematodes, in addition to bacteria, ingest metabolites in a similar way as has been observed in axenic cultures on artificial media (Nicholas 1962). If this is the case in soil, then the nematodes would act as competitors to the bacteria in addition to the role as predators.

Besides their influence on the total numbers of AO- and FDA-stained bacteria the nematodes also affected the frequency of the different morphological groups. The almost total dominance of cocci in the nematode free series at the end of the experiment could indicate a

reduction of rods due to competitive exclusion. By contrast, the relatively larger proportions of rods in the presence of nematodes may indicate reduced competition between the different bacterial populations, due to predation pressure exerted by the nematodes (Powell 1980). Thus, it is possible that the presence of a predator increased the diversity on the prey trophic level. This is also often found by theoretical considerations of predator-prey systems (e.g. Jost et al. 1973).

In other laboratory experiments using podzolic soil as substrate (Rosswall et al. 1977, Bååth et al. 1978, 1981a, Clarholm et al. 1981) the numbers of nematodes were many times higher than field values (Sohlenius 1979). In these experiments Acrobeloides nanus was a dominating species among the nematodes. Although high, the nematode numbers did not increase to the same level as they did in the present experiment. Our extremely high densities could be a result of liming of the soil mixture, which increased pH and probably increased release of nutrients from the soil. This stimulated bacterial growth, which was reflected in the high nematode abundance.

It is difficult to extrapolate findings from laboratory experiments to the field situation. Lundgren and Söderström (in press) found a positive correlation between FDA-stained bacterial numbers and nematode numbers in a field study. This relationship was not observed in the present laboratory experiments. To further illucidate the relationship between these organisms, other factors have to be taken into consideration. For example, other bacterial feeding organisms

probably influence this interaction. Clarholm (1981) has shown that protozoa are important in regulating bacterial populations in a pine forest soil. The presence of a third trophic level, i.e. a predator on the nematodes, is also of importance.

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Table 1. Biological parameters (mean values ± standard error) estimated at two occasions in the second experiment. Figures indicated with the same letter differ significantly ($p < 0.05$, Tukey-Kramer method based on 2-factor ANOVA). The ANOVA of respiration measurements was based on log-transformed data. In the treatment with nematodes 18 replicates were sampled each time, while in the treatment without nematodes 3 replicates were sampled at 22 days, and 4 replicates at 65 days.

Time (days)	22		65	
Treatment	with nematodes	without nematodes	with nematodes	without nematodes
Respiration ($\mu g\ CO_2\ g^{-1}dw\ h^{-1}$)	26 ± 2	13 ± 6	9.0 ± 0.6[a]	6.2 ± 0.2[a]
FDA-stained bacterial numbers ($x\ 10^{-9}\ g^{-1}dw$)	3.22 ± 0.11	2.89 ± 0.25	2.26 ± 0.10[b]	3.42 ± 0.08[b]
AO-stained bacterial numbers ($x\ 10^{-9}\ g^{-1}dw$)	7.06 ± 0.26	6.68 ± 0.43	4.96 ± 0.26	5.98 ± 0.69
Nematode numbers ($x\ 10^{-3}\ g^{-1}dw$)	2.28 ± 0.38	0	9.15 ± 0.60	0

Table 2. A carbon budget was calculated for the first experiment. Nematode data was calculated according to Klekowski et al. (1972) and Sohlenius (1979). The bacterial efficiency value was assumed to be 0.45. All figures are given in mg C g^{-1} dry soil.

	First period 1 - 11 days		Second period 17 - 54 days	
	nematodes with	without	with	without
Total cumulated CO_2-evolution	4.40	4.81	5.63	3.83
Total nematode production	0.0003	0	0.30	0
Total nematode feacal production	0.0009	0	0.82	0
Total nematode CO_2-evolution	0.001	0	0.94	0
Total nematode consumption	0.002	0	2.04	0
Total bacterial CO_2-evolution	4.39	4.81	4.69	3.83
Total bacterial production	3.60	3.94	3.84	3.14

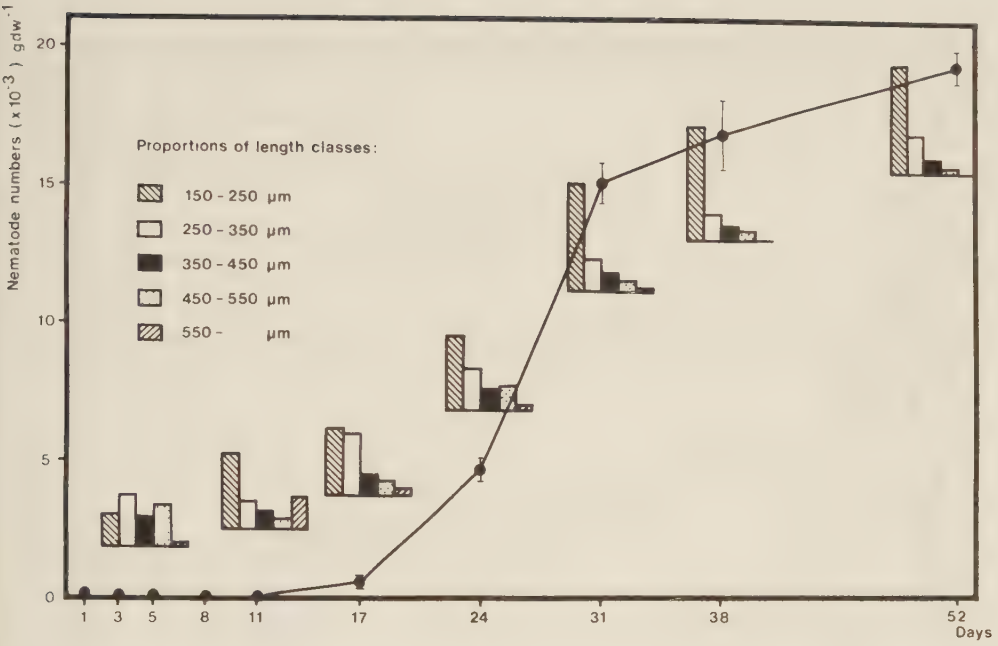

Figure 1. Development of the nematode population in the nematode series of the first experiment (mean nematode numbers of three replicates ± standard error). The histograms show the distribution of the nematodes in different length classes at different times.

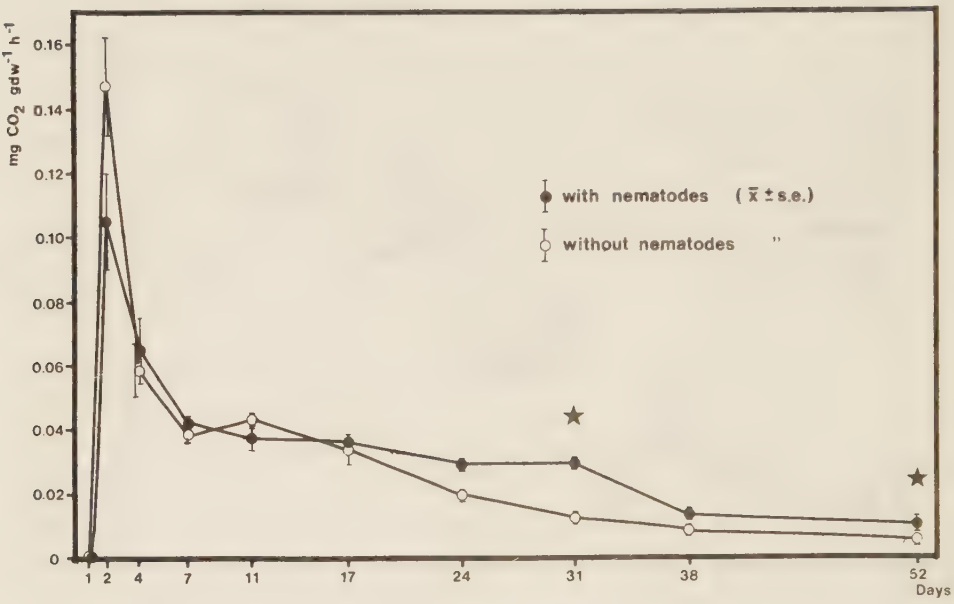

Figure 2. Respiration rates of the two treatments with and without nematodes in the first experiment (mean values of three replicates ± standard error). The stars indicate significant difference ($p < 0.05$, Tukey's honestly significant difference method, based on 2-factor ANOVA on log-transformed data) between the two treatments at those occasions.

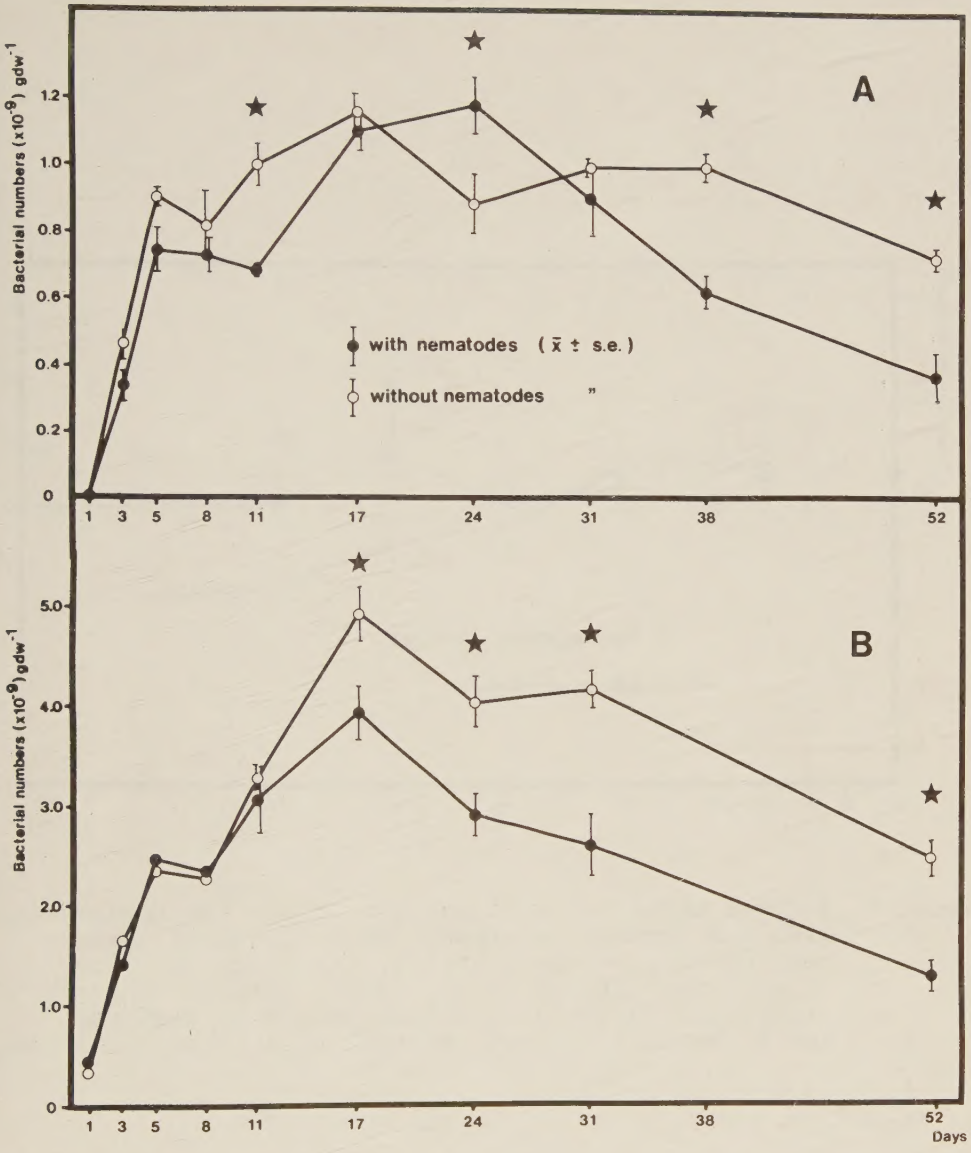

Figure 3. Numbers of bacteria in the two treatments with and without nematodes in the first experiment (mean values of three replicates ± standard error). (A) shows the FDA-stained bacterial numbers and (B) the AO-stained bacterial numbers. The stars indicate significant difference ($p < 0.05$, Tukey's honestly significant difference method, based on 2-factor ANOVA) between the two treatments at those occasions.

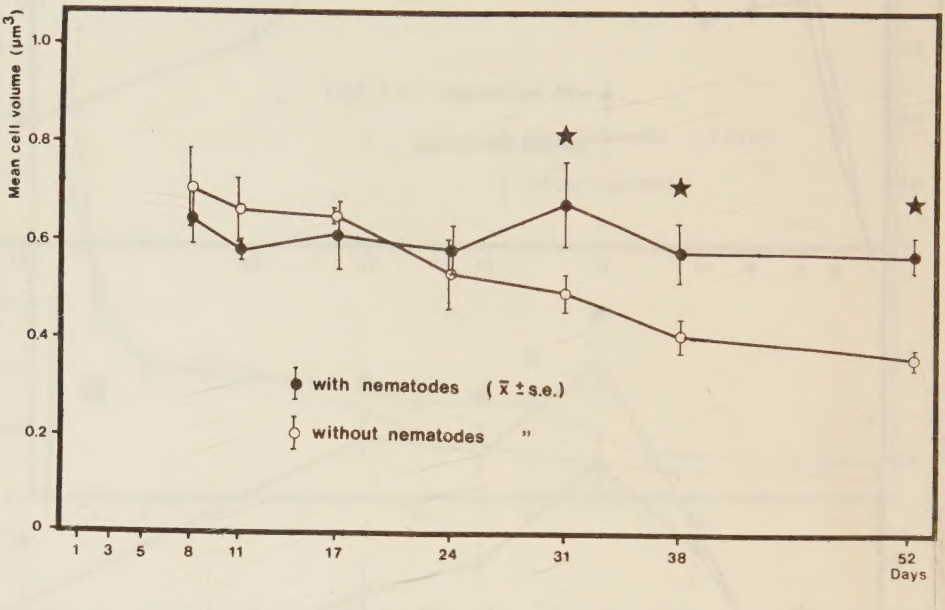

Figure 4. Average bacterial cell volumes of the two treatments with and without nematodes (mean values of three replicates ± standard error). The stars indicate significant difference ($p < 0.05$, Tukey's honestly significant difference method, based on 2-factor ANOVA) between the two treatments at those occasions.